NASA-CR-66703

A STUDY OF THE POSSIBLE MOVEMENT OF MICRO-ORGANISMS THROUGH SMALL ORIFICES

FINAL REPORT

AVSSD-0219-68-RR

Prepared Under Contract No. NAS 1-7277

Avco Space Systems Division

Lowell, Massachusetts, 01851

For

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

FOR NASA INTERNAL USE ONLY

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A STUDY OF THE POSSIBLE MOVEMENT OF MICRO-ORGANISMS THROUGH SMALL ORIFICES

BY AVCO SPACE SYSTEMS DIVISION
LOWELL, MASSACHUSETTS, 01851

INTRODUCTION

This final report presents the technical approach and results of work performed under Contract NAS 1-7277.

The problem statement of the required basic research task was to investigate the possibility of microorganism transport through a small hole or fissure in a bio-barrier over the various regimes of fluid flow. The research objective was to implement devices for performance of required tests and to provide sufficient test data to permit an intelligent forecast as to whether bacteria can or cannot migrate through small holes in a bio-barrier.

The subject problem is applicable to flight hardware such as sterilization canisters and ground facilities such as the NASA/LRC MAST (Model Assembly Sterilizer for Testing) and its succeeding larger facilities for launch operations.

The existence of violation passages through a bio-barrier can result from microscopic manufacturing defects, incomplete sealing of functional passthroughs in a bio-barrier, or mechanical and micrometeorite puncture.

The specific investigation carried out under this contract has addressed one of the necessary considerations concerned with an a priori bacterial violation of a bio-barrier. The work reported herein was restricted to the violation challenge by bacteria approaching at low velocity (less than 10 ft/sec.) a small round hole issuing an opposing gaseous fluid flow at velocities from zero to sonic. Postulation of conditions that would cause bacterial approach velocities orders of magnitude higher than utilized in this investigation can quite easily be made. Similarly, as only quasi-static force fields have been considered in this work, dynamic or oscillatory force fields that may result in violation can be postulated when consideration is made of a typical vehicle mission profile.

The applicability of the results obtained in this program must be bounded to assist the NASA in defining the need of additional work concerned with the general problem and also minimize extrapolation of the results by the community to areas involving physical parameters beyond the regimes considered.

First, the results pertain to small round holes with diameter to length ratios between 0.1 and 1.5 for pressure ratios across the hole ranging from 1 to 50. As can be seen from the results, the validity of the data is best for pressure ratios near 1 and ambient pressures near atmospheric. The results are substantially less valid at high pressure ratios and ambient pressures in the vacuum regime.

Second, the results pertain to velocities of motion of the challenging particle of less than 10 ft/sec. As the highest estimate of limiting velocity of a typical spore of Bacillus globigii is 4 ft/sec. at 1 atmosphere ambient, the results are most pertinent in the free sea level pressure environment and are substantially non-inclusive of velocities that can conceivably be achieved in the space vacuum environment.

Third, the results are concerned strictly with gas pressure fields and the work has been performed in a manner that has hopefully eliminated (with the exception of gravitational) other force fields such as electrostatic and electromagnetic fields.

This report includes discussion of the test techniques employed, the implementation of test devices, presents the test data and concludes with a violation model for the near atmospheric pressure regime.

EXPERIMENTAL TECHNIQUES AND PROCEDURES

The research goal of this program was implemented through investigation of the problem in the following two ambient pressure regimes:

- Determine if microbial penetration through a 0.001 inch-diameter hole was possible against various fluid flows at low pressures analogous to conditions prevailing for a sterilization canister in vacuum flight.

- Determine if microbial penetration through 0.006, 0.010 and 0.020 inch-diameter holes was possible against impeding differentials in pressure of up to 4 inches of water for ambient pressures near one atmosphere.

The means utilized to detect bacteria that violated a test hole uniformly employed direct visualization of stained organisms using fluorescence microscopy.

The basic procedure used in the tests of this program was to assemble two chambers which were connected by a small, single orifice. In the first chamber, called the inoculation chamber or the main chamber, the microbes were dispersed in the ambient gas which had a pressure P_1. The second chamber (the violation chamber or test cell) was sterile at the start of each test. At the end of each test it was searched for violations using the technique of fluorescence microscopy. The pressure in the second chamber is designated P_2 and in general $P_2 > P_1$. In proof tests the reverse was true ($P_1 > P_2$).

Low Pressure Tests

A series of tests were carried out in which P_1 was the order of 10μ (10^{-2} torr) and the orifice diameter was 0.001 inch. For these tests a four (4) inch diameter vacuum tank was used as the main chamber. Mounting flanges were provided for attaching the test cell in any of three orientations with respect to gravity. Figure 1 is a photograph of the low pressure test setup. In the figure the violation chamber is oriented on the top flange in the manner which gravity impedes violation. Both chambers were evacuated by mechanical vacuum pumps through valves, and the pressures were measured with thermocouple gauges.

The violation chamber was specifically designed for this program. Two design requirements, that it be vacuum tight and that it be made of flat pieces of glass easily scanned in the microscope, seemed to be contradictory. Our designs to overcome this problem are shown in Figure 2. The test cell is actually a double chamber; the outer wall constitutes the vacuum seal and the inner components are the glass plates which collect the violating organisms. In Figure 3 this inner glass structure is shown more clearly. The glass end plates were grooved to receive the hexagonal array of side plates. A compression spring (not shown in Figure 3) kept the assembly together.

Considerable effort was spent investigating techniques of creating and maintaining the microbial cloud in the inoculation chamber. Since the air pressure in this chamber for these tests was low there was no resistance to fallout of the cloud due to gravitational forces on the particles. In the dispersing device which was developed this fallout loss was partially overcome. Dried spores were placed on a rubber diaphram stretched across

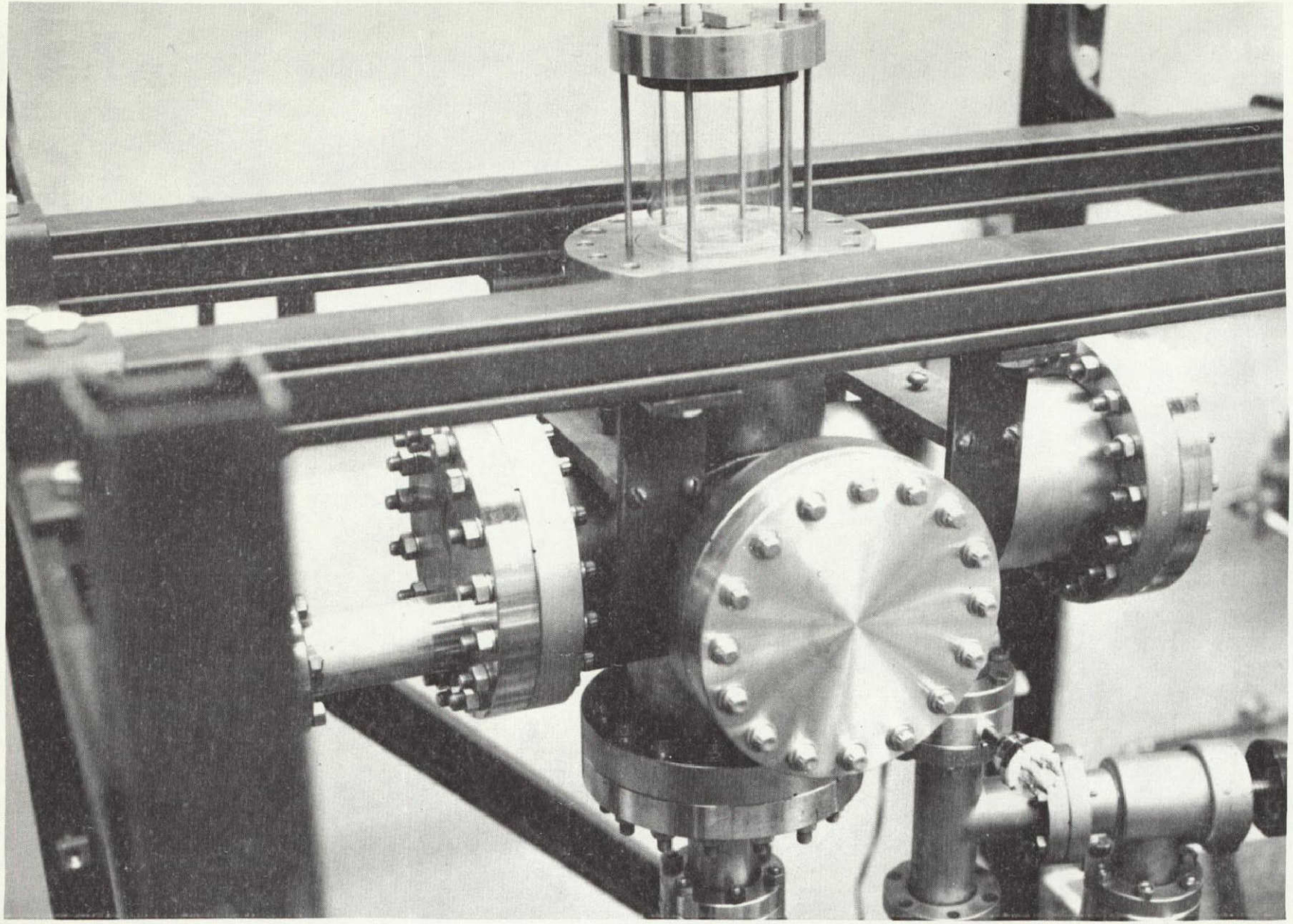
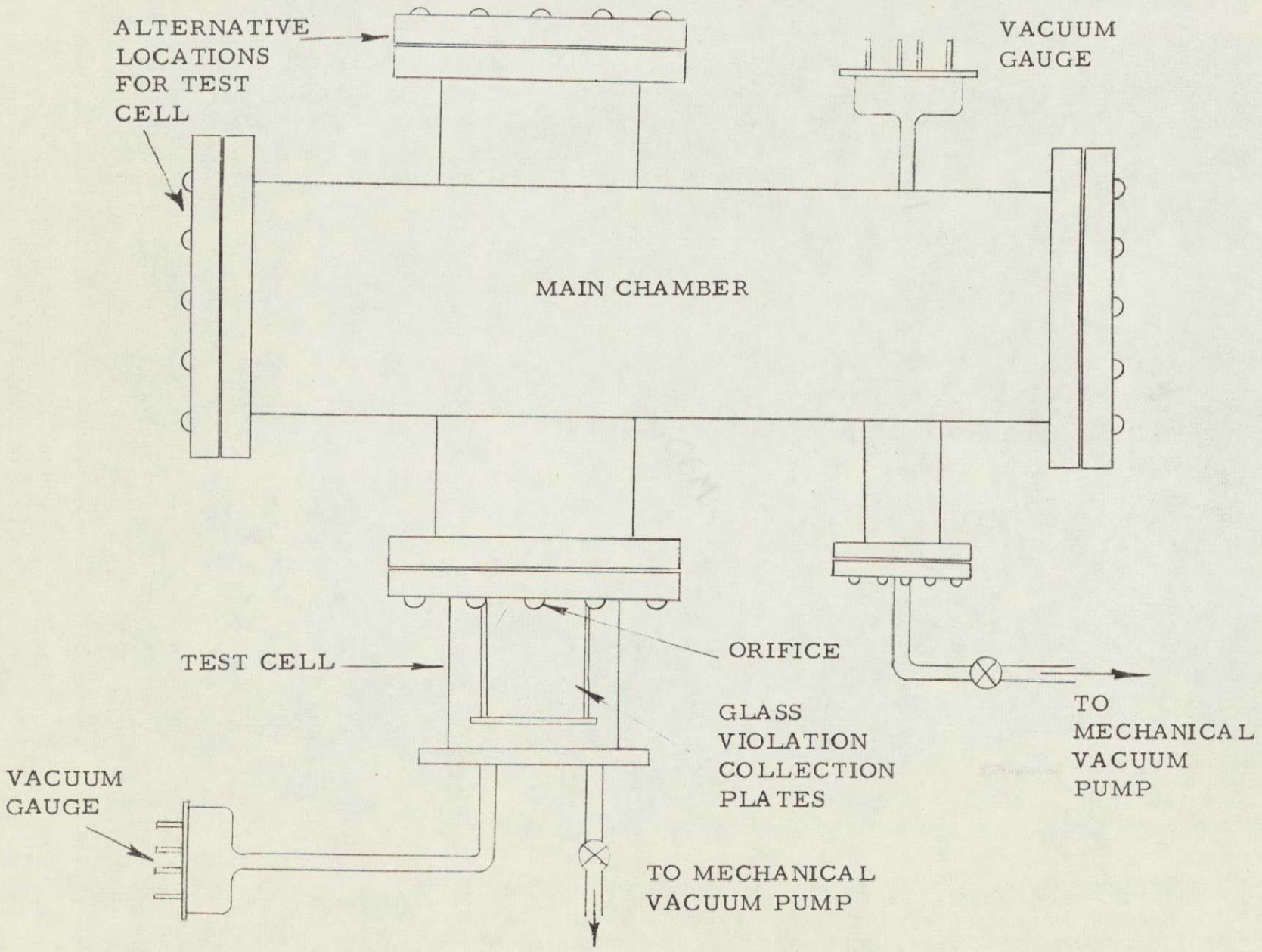


FIGURE 1. - FOUR INCH MAIN CHAMBER WITH
ATTACHED TEST CELL

FIGURE 2
DIAGRAM OF TEST SYSTEM



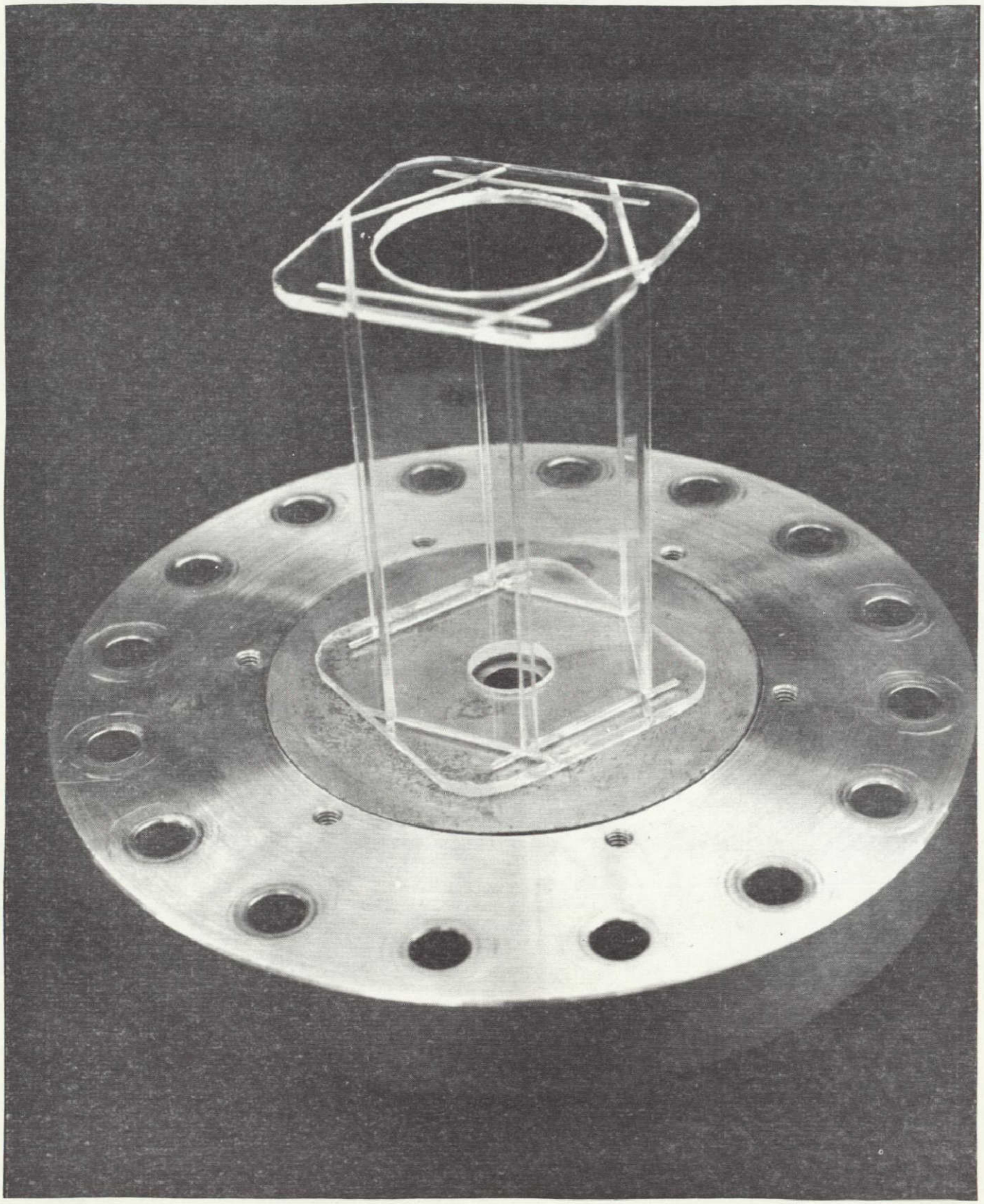


FIGURE 3. - TEST CELL WITH OUTER
CYLINDER REMOVED

the lower end of a 3/4 inch diameter, 1/2 inch long glass tube. The center of the diaphram was attached to an iron armature which was driven by a solenoid. The cloud was formed when the organisms were thrown upward by the vibrating diaphram. Some of the fallout was recollected on the vibrating diaphram in the glass tube and recycled. This device is shown in Figure 4 as it appeared following a test. Clumps of organisms of varying sizes can be seen coating the device and the walls of the main chamber. (The problem of clumping will be discussed in a later section).

Tests At One Atmosphere

Conceptually, the second series of tests was the same as those just described. Because of the different pressure regimes the details are quite different. A photograph of the equipment is presented as Figure 5 and Figure 6 is a schematic of the test setup. The main chamber, a 14" diameter bell jar, rested on a base plate beneath which the test cell was attached. In these tests gravity (down) was always parallel to the cylinderical axis of the orifice and directed from the main chamber to the test cell.

The test cell used in the first phase tests was modified slightly for these tests. It had been found that the array of glass plates previously used was unnecessarily complex. A single 35 x 10 mm plastic Petri dish was substituted and this change greatly reduced the time required for microscopic scanning.

The vacuum pumps previously used were not required in these tests. The main chamber pressure, P_1, was one atmosphere since it was open to the room through a vent (Figure 6). (The vent discharged into a flask containing Cidex, a chemical disinfectant, to minimize accidental room contamination). The pressure P_2, in the test cell was maintained between zero and four inches of water higher than P_1 in these tests. This was accomplished by connecting the cell to the laboratory compressed air supply through an air pressure regulation system (Figure 7). Regulation was accomplished using a diaphram type regulator (Hoke type 804A10). This means of regulation was adequate for pressure differentials greater than two inches of water. For regulation at lower pressure differentials the reference pressure in the regulator was reduced using a water aspirator. The supply air was dried with calcium chloride just prior to entering the test cell. The pressure differential between the main chamber and the test cell was measured directly with a water manometer. The manometer could be read with an accuracy of about ± 0.2 inches and it was found that fluctuations in the pressure differential did not exceed that tolerance. In the proof tests (reversed flow, $P_1 > P_2$) the vent and inlet connections were interchanged.

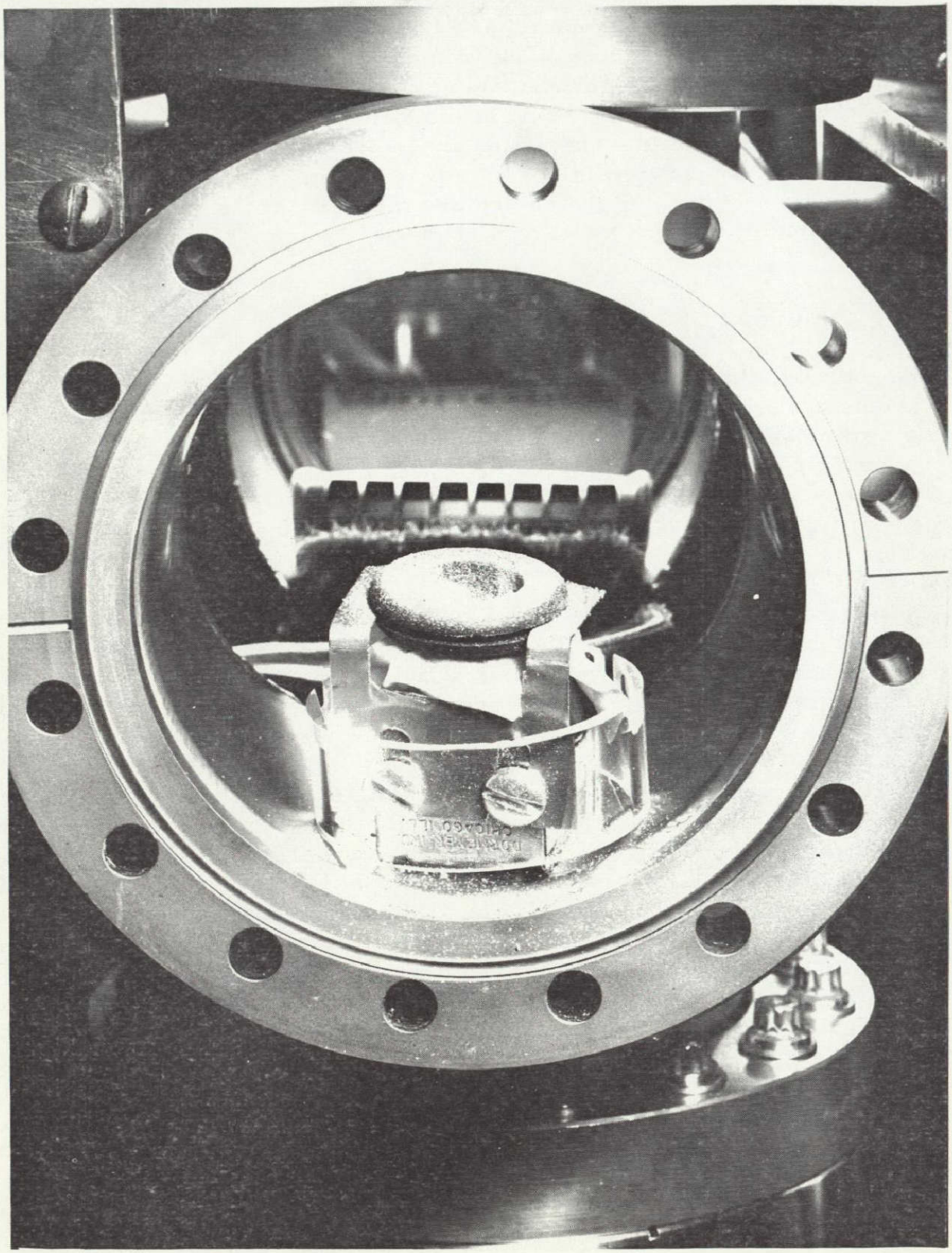


FIGURE 4. - DISPERSION DEVICE FOLLOWING
LOW PRESSURE TEST

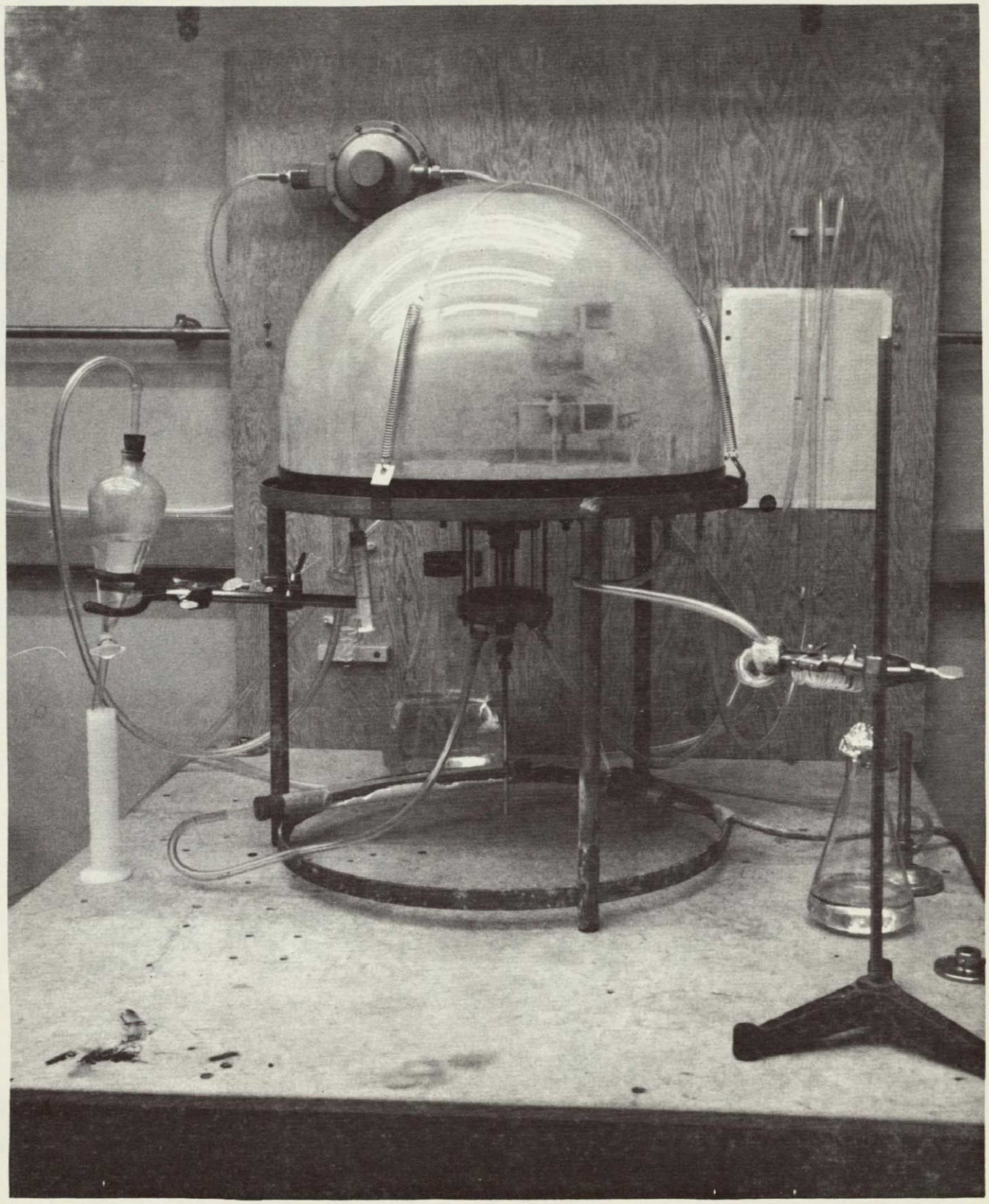


FIGURE 5. - BELL JAR SYSTEM WITH
ATTACHED TEST CELL

FIGURE 6

1-1689 SCHEMATIC DIAGRAM OF BELL JAR SYSTEM

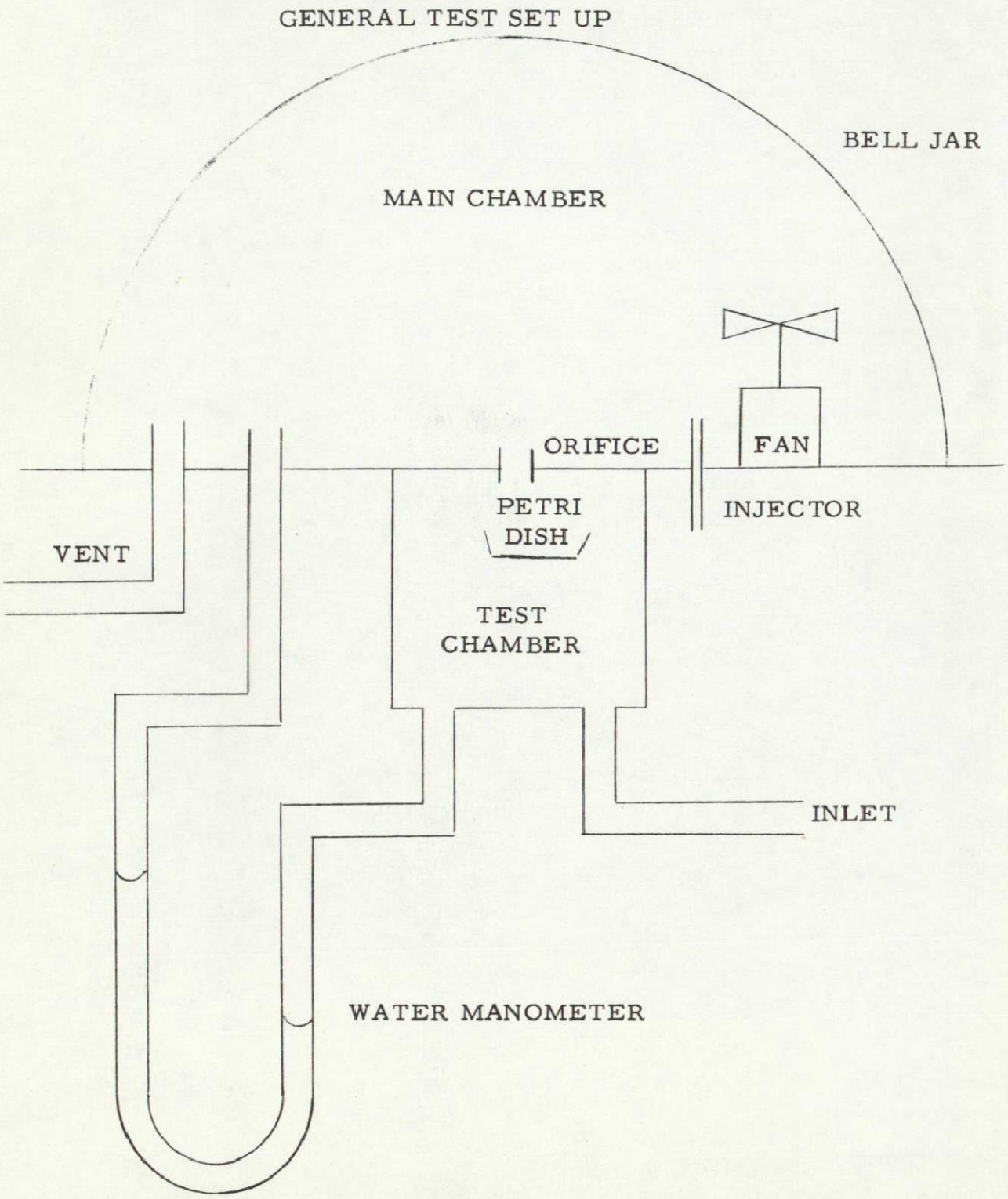
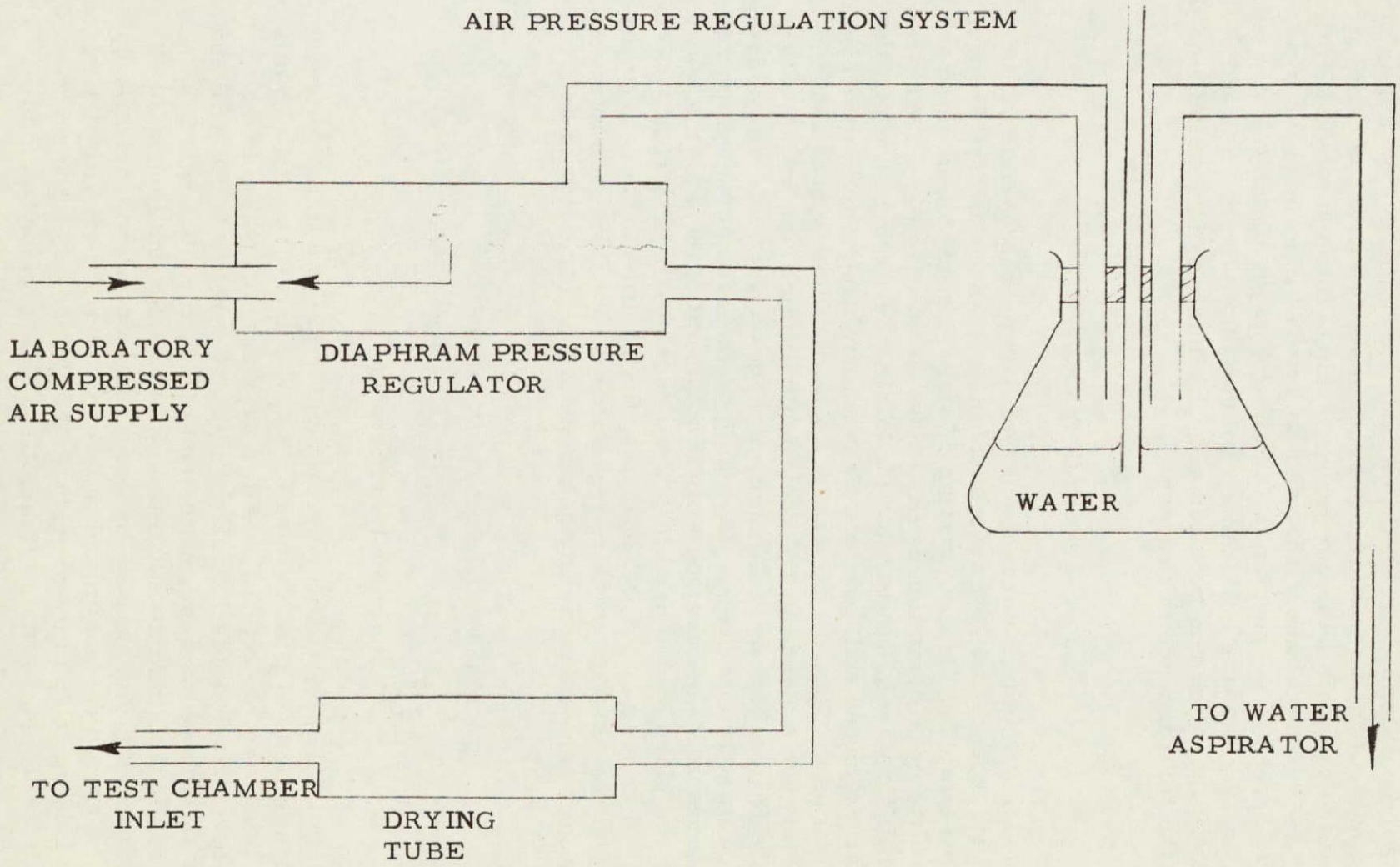


FIGURE 7

AIR PRESSURE REGULATION SYSTEM



Because the pressure in the chamber was one atmosphere and not a partial vacuum it was possible to use a different and more effective organism dispersal technique. A quantity of dried organisms was introduced at desired time intervals to the main chamber from a syringe through a hypodermic needle. A blower fan within the chamber was used to increase the time that the cloud stayed in suspension. The density of the cloud was measured during each test by drawing a measured volume of gas from the main chamber through a Millipore filter and then counting the number of organisms on the filter using the fluorescence microscope.

Fluorescence Microscopy

The Leitz Model Ortholux Fluorescence Microscope was used for determination of the fluorescence level of specimens stained with acridine orange. The instrument system used for examination of stained microorganisms consisted of an ultraviolet light source (HB-200W osram lamp), a UV transmitting filter of a specific wavelength band, a darkfield microscope in which the specimen was examined and a visible light transmitting filter in the eyepiece of the microscope to allow the observer to see the fluorescence induced. Stained spores when exposed to UV radiation will fluoresce with a color specific to the fluorescent dye. Acridine orange-stained microorganisms will fluoresce mist red or green, depending on the dye concentration and age of microorganisms. Thus, by examining specific areas of the test cell, (either the glass slides used in low pressure tests or a small plastic Petri dish used in near atmospheric tests), the position of the tracer organism could be revealed. Unstained microorganisms do not fluoresce in the red or the green so they are easily distinguished from the tracer organism.

MATERIALS AND METHODS

Biological Preparation

Bacillus globigii (B.g) spores were grown and harvested in the following manner: A four mm loop of pure culture of B.g was inoculated into 75 mls of sterile trypticase soy broth (TSB). After 24 hours incubation at 32°C, 0.5 ml of the cell suspension was inoculated into 250 mls of sterile TSB and incubated at 32°C for 24 hours. At the end of this time 0.1 ml of the cell suspension was inoculated into 500 mls of sterile TSB and incubated as before. Periodically, the suspension was examined microscopically by Gram stain. The culture was mixed thoroughly and 0.5 ml aliquots were spread over 150 mm x 25 mm intergrid Petri plates containing trypticase soy agar with manganese sulfate (10mg/liter) and calcium chloride

(40mg/liter) as additives. The Petri plates were incubated at 32°C for 5-7 days or until that time necessary for maximum sporulation to occur. Microscopic examinations were made daily to ascertain the degree of sporulation.

The spores were harvested with sterile cold (4° - 10°C) deionized water. The pooled spores were then centrifuged at 8000 rpm for 15 minutes. The supernatant was poured off and the spores resuspended in cold deionized water. The rinse procedure was repeated five times. After the fifth wash the supernatant was decanted. The B. g spores were then suspended in 0.5% acridine orange fluorescent stain prepared in veronal acetate buffer, pH 5.3. The spores were stained for four minutes, and then centrifuged at 8000 rpm for five minutes. The residual acridine orange was decanted. The spores were then resuspended in veronal acetate buffer, pH 5.3, and centrifuged again at 8000 rpm for five minutes. The wash and rinse procedure was repeated three times to remove excess dye from the spore suspension. After the final rinse procedure, the organisms were placed in a desiccator containing silica gel and were dried. This drying procedure proved to be unsatisfactory in that the dried spores tended to form large aggregates. This problem of clumping was alluded to earlier in the report. The problem being studied was the possible migration of organisms through small holes. If clumps of organisms larger than the hole were used, the hole would become plugged or partially so, thus changing the flow parameters of the experiment. It was found that drying of spores over silica gel produced excessively large clumps which could not be tolerated and after some experimentation, a freeze-drying technique was found which produced an acceptable spore powder.

Following the staining procedure a slurry of B. g spores were put in glass jars, frozen, and then placed in a VirTis Freeze-Dryer. A vacuum was drawn and, as the samples warmed, the water sublimed and was collected in a dry ice-methanol cold trap. All moisture was removed from the frozen spore cakes without melting and left the desired finely divided spore powder.

After freeze-drying, the stained B. g spores were stored in a desiccator in a dark container over NaOH pellets. A polonium source was placed with the spores unitl their experimental use. The alpha ionization from this source prevented the build-up of electrostatic charge which would have interfered with spore dispersion.

Test Cell Preparation

The techniques of this program required that the glass plates or the small Petri dish of the test cell be clean for two reasons. First, these surfaces

must be free from the tracer organism so that false violation would not be reported and second, the surfaces must be as free from dust particles as possible to prevent light scatter in the microscope which greatly increases the difficulty of observation. In the initial cleaning procedure the glass plates were:

(1) washed in hot Alconox,
(2) rinsed in sterile deionized water,
(3) soaked in chromic acid for 18 hours,
(4) rinsed in sterile deionized water and dried.

This procedure was modified during the course of the study. It was apparent that most of the slides still possessed an oil slick. The slides were then cleaned in the following manner:

(1) The slides and cover slips were placed in the thimble of a Soxhelett extractor.
(2) Diethyl ether was added and extracted for three hours.
(3) The ether was removed and the process was repeated using acetone followed by isopropyl alcohol.
(4) Following extraction, the slides were rinsed in filtered, sterilized, distilled water.
(5) The slides were placed in chromic acid for 12-18 hours.
(6) At the end of the chromic acid bath the slides were rinsed with filter-sterilized deionized water.
(7) The slides were dried with acetone and stored in a dry container.

The small Petri dish was supplied clean by the manufacturer and the above procedures were not necessary.

Detailed Test Procedure

Low pressure tests. - After the test cell and its glass inserts were cleaned, the cell was assembled in a horizontal laminar flow work station. The diaphram-type dispersion device was loaded with dry spores and the test cell was mounted on the main chamber flange giving the desired gravitational orientation.

The procedure for evacuation of the two chambers was carried out with great care. Pump down of the test chamber was not begun until the main chamber was evacuated to a pressure lower than would be used in the actual test. This was done to assure that flow through the orifice was always in

the direction of the main chamber and at a rate higher than that to be used in the test. After the test cell pressure reached the desired level and the main chamber pressure was adjusted, the dispersion vibrator was energized. Scattered light from a small helium-neon laser showed the extent and density of the spore cloud in a qualitative way. At the end of the test, air was re-admitted to the two chambers in a manner that maintained the desirable ΔP so as not to invalidate the test. It should be noted that the test cell was made with a face seal, O-ringed plunger, which could be used to seal the orifice during the pumpdown and bleed-in periods had there been evidence of violations during those events. This step had the disadvantage that it deposited vacuum grease on one of the glass collection surfaces making that area unavailable for microscopic examination. As the results will show, the use of this plunger was not necessary.

Atmospheric tests. - The apparatus for these tests has been described in the section discussing tests at one atmosphere. After sterile assembly of the test cell, it was positioned beneath the base plate of the main chamber. An O-ring seal was used. This step was carried out without agitation of the organisms in the main chamber. After the air supply was turned on and ajusted to give the desired differential pressure, the circulating fan within the main chamber was turned on and the challenge spores were injected from a 10cc hypodermic syringe. The concentration of the aerosol was monitored by extracting 100 mls of air from the main chamber through a clinical-type Millipore Hospital Monitor. The procedure was as follows:

(1) The monitor was type AA Field Monitor containing a white grid-marked membrane filter which retains bacteria and is equipped with a Millipore screw limiting orifice.
(2) The monitor was connected to the main chamber by means of Tygon tubing.
(3) The B. g aerosol was generated, allowed to stabilize and then sampled by drawing the required volume of aerosol sample through the monitor membrane.

It was found that the aerosol did not remain suspended for the duration of the tests even with the fan operating. Because the spores were introduced to the main chamber from an external syringe, it was a simple matter to add additional charges as required. This was done periodically in each test. Separate tests were performed as will be described in the section on the dispersion of B. g during atmospheric tests which permitted analysis of the effective challenge obtained by this procedure.

At the end of the test period, the fan was stopped and the test cell removed from the main chamber for assay.

Microscopic Scanning

Upon the completion of each test, the test cell was removed from the main chamber and disassembled. As the turbulence of a burner flame might dislodge the violating spores on the glass plates, the standard technique of flame heat-fixing was not used. Each of the glass slides from the test cell was covered with a second sterile slide which had been cleaned by the same procedure used for the test cell plates. For the tests using Petri dishes, a clean cover was placed on the dish as it was removed from the cell.

Scanning of the glass plates was a tedious effort, especially because of the low occurrence of violations. For this task, the Avco Quantitative Fluorescence Microscope was employed. By using the UV/TV microscope, it was possible to quantitate in millimicrolumens the fluorescence level of individual microorganisms. The Avco Quantitative Fluorescence Microscope has the following components which are designated in Figure 8.

A. - A high intensity 1000 watt Hg-Xe source.
B. - A grating UV and visible light monochromator.
C. - A primary filter which is used to narrow the wave band of the UV light to irradiate the specimen.
D. - An apochromatic UV-visible light microscope with matched quartz optics utilizing bright field illumination.
E. - A secondary filter system which transmits only in the region of fluorescence from the stain. The secondary filter is used only in the microspot sampler beam to select a bandwidth for the quantitative photometric measurement.
F. - A UV vidicon camera which receives the entire field in the UV or near-UV region for visualization of stained and/or unstained cells.
G. - The microspot scanner, approximately 1/2 μ^2 in area or larger, used to select specific areas of the specimen for the quantitative photometric measurement.
H. - A photomultiplier which provides a quantitative read-out of the light intensity in the spectral region determined by the secondary filter (E).
I. - A TV monitor which displays not only a UV absorption image of cells, but also provides a visual indication of the position of the microspot scanner showing the area being quantitatively examined.
J. - A cathode-ray-oscilloscope which provides additional and simultaneous quantitative information on the UV absorption profiles.

A block diagram of the microscope is given in Figure 9.

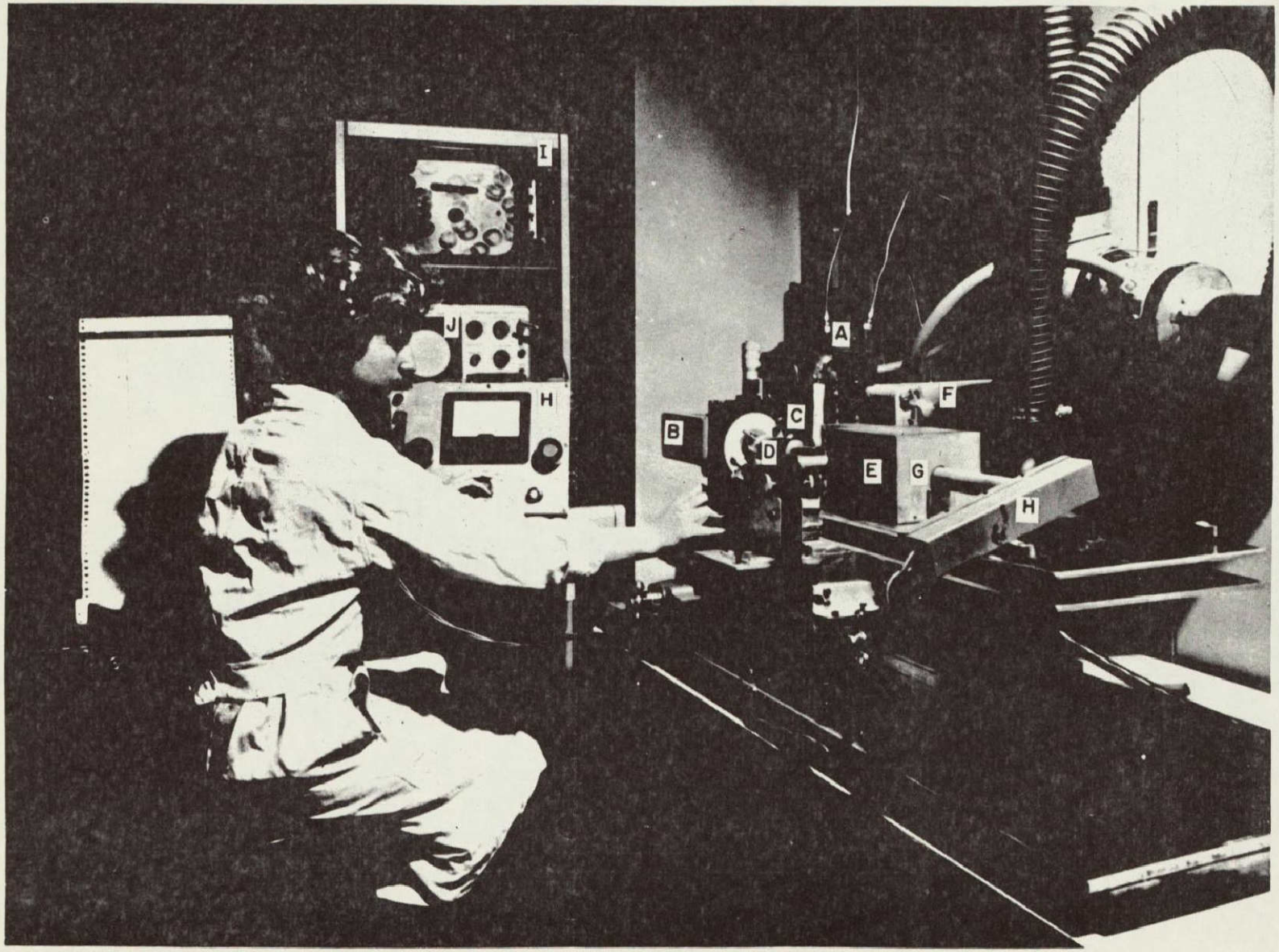


FIGURE 8. - PHOTOGRAPH OF THE AVCO QUANTITATIVE UV/TV MICROSCOPE

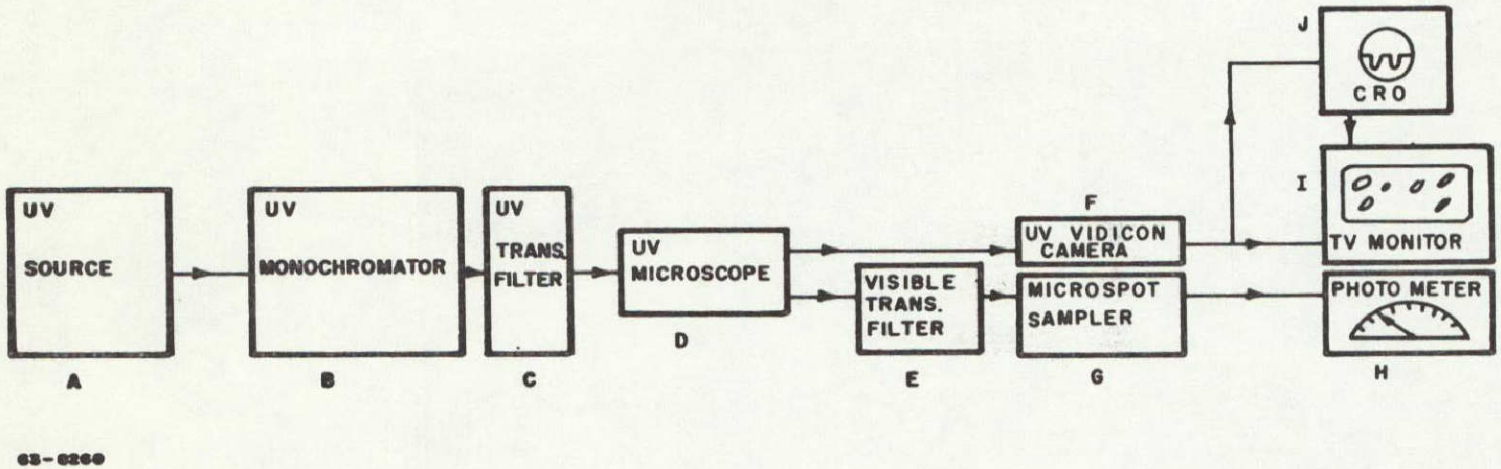


FIGURE 9. - BLOCK DIAGRAM OF AVCO QUANTITATIVE FLUORESCENCE MICROSCOPE

In the atmospheric tests, it was not necessary to use the UV/TV microscope. Each Petri dish was scanned under low power using a Leitz Fluorescence Microscope. When clumps were observed, the magnification was increased to 250 times. Exact counts of the penetrating microorganisms were made whenever possible. If the penetrating organisms were greater than 10^3 the count was scaled up by an area distribution formula and by representative counts in known areas.

A technique for sampling the main chamber aerosol has been described. After a sample was taken, the monitor was removed from the chamber, disassembled and the membrane removed for assay.

The membrane was cleared with immersion oil and mounted on the microscope stage. Using an ocular micrometer in one eyepiece (five mm square) and a stage micrometer, the measuring eyepiece was calibrated. A statistical particle counting method was used to count the fluorescing particles on the filter. Areas were scanned by manipulating the stage so that particles to be counted passed under the ocular micrometer scale. The particles were sized as part of the counting procedure. Only the maximum dimension of the particle was regarded as significant. For particles improperly oriented relative to the ocular micrometer scale, an estimate of the dimension was made. Calculation of the total number of particles of a given size was done as follows:

$$P_t = N_t \times \frac{900}{n \times A_f}$$

where,

P_t = total number of a given size range on the filter,
N_t = total number of particles counted in n unit areas,
n = number of unit areas counted,
A_f = unit area in square millimeters,
900 = total effective filter area in square millimeters.

Spore Clump Size Study

Early in the program, as part of the development of the diaphram dispersion device and the freeze-drying procedure, a study of clump size was made. In Figure 4 (the photograph of the diaphram dispersion device) a slotted glass ring can be seen. The slots in this ring were used to support 1 x 3 inch microscope slides used in the spore clump size study. The slides were supported on end leaning slightly outward. The procedure was as follows:

1. Clean, sterile slides were placed in the support ring.
2. An orifice test was conducted as described on page 7.
3. After the test, the slides were removed and heat fixed.
4. The spore clumps were examined microscopically and compared with a piece of 0.001 inch-diameter wire which was placed in the field of view.
5. A count was made of clumps larger than and smaller than 0.001 inch in the upper and lower halfs of each slide.

Dispersion of B. globigii During Atmospheric Tests

The purpose of the experiment was to determine the aerosol concentration of the B. globigii spores in the main chamber against height and time. This was done to validate the measurements of aerosol density made as part of the atmospheric tests.

A 1/4" diameter piece of glass tubing approximately 10 inches long, bent at a right angle was inserted into the main chamber (bell jar) at the orifice position. A Millipore monitor was connected to the outside end of the glass tubing. The monitor was then connected to the top of a separatory funnel.

The B. g spores were injected into the main chamber only once by using a 10 cc syringe and an 18 gauge needle. The fan was turned on to keep the organisms in suspension. At the required time periods, (one minute, three minutes, six minutes, 12 minutes, and 20 minutes) 100 mls of water was removed from the separatory funnel. This in turn removed 100 mls of aerosol on to the field monitor. This experiment was repeated at the times indicated with the glass tubing at 0, 1/2 inch, one inch, and 1 1/2 inches above the floor of the chamber.

After the 100 mls of air and particles were impacted on to the field monitor, the monitor was dismounted, the filter removed and placed on a 2" x 2" glass slide, coated with immersion oil and placed on the Leitz fluorescent microscope for biological evaluation. Examination of the filter was carried out as described as part of the atmospheric tests.

Counts of the fluorescing B. g spores were made to determine particulate count per unit volume of the aerosol sampled.

After the measuring eyepiece was calibrated, counts were made by scanning 10 areas. The number of particles in each unit area of the reticule was counted. The total number of particles was calculated using formula (1) on page 19.

To calculate the standard deviation, the mean of the 10 counts was obtained by dividing the sum of the ten counts by the number of counts ($\overline{X}$). The variation of each measurement from the mean ($X - \overline{X}$) was determined. These variations were then squared to convert all of the minus values to plus $(X - \overline{X})^2$.

To determine the standard deviation, the sum of the squared variation from each mean was divided by the number of counts. The square root of that particular number determined the standard deviation.

Comparison Of The Fluorescence Levels Of Desiccated B. globigii Spores With Freeze-Dried B. globigii Spores

By using the Avco UV/TV Fluorescent Microscope it was possible to quantitate in millimicrolumens the difference between B. globigii spores grown, stained and freeze-dried and old, unstained, freeze-dried spores, reconstituted in sterile distilled water, stained with 0.05% acridine orange, washed three times, centrifuged and desiccated over silica gel.

After each group of organisms was dried, they were dusted onto 1 x 1.5 inch microscope slides. Cover slips were added and sealed with "Duco" cement.

Following slide preparation fluorescence levels were determined by scanning for individual cells only. Before the smears were examined, the fluorescent microscope system was standardized at 450 m μ and aligned.

TEST RESULTS

Low Pressure Tests

The intent of the low pressure tests was to assess the possibility of recontamination of a lander within the sterilization canister for the case of small leaks in a bio-barrier. The pressure regime approached space vacuum. A leak of this kind was simulated by 0.001 inch diameter orifice between two chambers as has been previously described.

Violation Tests. - Tests were carried out for a number of different pressures in the test cell and in the main chamber. For the preliminary case, to demonstrate transport by reverse flow, the test cell pressure was less than the main chamber pressure. The results for these tests

are listed in Table I. The technique of drying, the position of the test cell and the pressures are indicated for each test. As can be seen, few if any violations were found.

One forward flow test ($P_2 > P_1$) is also listed in this table. No violations were found. Because of the very small number of violations found even in the more favorable condition of reverse flow, a re-evaluation of the program was undertaken with the cognizant technical personnel at LRC. (A parallel study, carried out at atmospheric pressure resulted).

Spore clump size distribution. - Table II shows the concentration and distribution of B. g spores dispersed in the vicinity of the test cell orifice by the vibrator dispersion device. These data were obtained by counting the organism fallout collected on 1 x 3 inch glass slides. In Figure 4 a slotted glass ring is shown surrounding the dispersion device. The glass collector slides were supported on end in these slots so that they leaned slightly outwards. The "top half" and "bottom half" of each slide were counted separately to obtain additional data on the spatial distribution of the larger clumps.

Two conclusions can be drawn from Table II. Between 80 and 90 percent of the clumps were generally found to be smaller than the 0.001 inch test orifice. This suggests that the probability of plugging the orifice with a large clump was finite but not large. Examination of each orifice following the tests confirmed this conclusion; occasional plugging was seen. The second conclusion is that the number of challenges in each test is small. This accounts in part for the small number of violations found in the reverse flow tests.

Atmospheric Tests

In those tests, three sizes of orifices were used: 0.006, 0.010, and 0.020 inch diameter. The differential pressure across an orifice ranged from zero to four inches of H_2O at a base pressure of one atmosphere.

For the atmospheric violation tests, a proposed test sequence was devised which would best establish the approximate threshold in pressure difference for violation. This plan is reproduced in Table III.

Before the actual test procedures for the determination of orifice violation were performed, proof tests were conducted to determine the acceptability of the system. The proof tests were run with four inches of water differential pressure (ΔP) between the main chamber and the test chamber with the main chamber at the greater pressure. The proof tests were run until violation

TABLE I
SUMMARY OF EXPERIMENTAL RESULTS

Test Number	After Freeze Drying B. globigii (Bg) stored over:	∝ Source in Vibrator M* Chamber	∝ Source in Desic-cator	Position of Cell	Pressure C* Pressure M*	Vibrator On While Injecting Air	Results
1	Dri-rite	No	No	Side	PC = 28 PM = 40	No	Negative
2	Dri-rite	No	No	Side	PC = 190 PM = 290	No	Negative
3	Dri-rite	No	No	Side	PC = 28 PM = 40	No	Negative
4	Dri-rite	No	No	Side	PC = 24 PM = 60-1000	No	Negative
5	Dri-rite	Yes	No	Side	PC = 29 PM = 60	No	Positive, hole plugged with Bg spores (plug of spores found inside hole, approximately 50 spores in hole).

(M* = Main Chamber)
(C* = Test Chamber)

TABLE I (Continued)
SUMMARY OF EXPERIMENTAL RESULTS

Test Number	After Freeze Drying *B. globigii* (Bg) stored over:	∝ Source in Vibrator M* Chamber	∝ Source in Desiccator	Position of Cell	Pressure C* Pressure M*	Vibrator On While Injecting Air	Results
6	Dri-rite	Yes	No	Side	PC = 19 PM = 105	No	Negative
7	Dri-rite	Yes	No	Side	PC = 28 PM = 96	No	Positive, 3 *Bg* spores found on cover slip, spores passed through hole.
8	Dri-rite	Yes	No	Top	PC = 18 PM = 54	No	Negative
9	Dri-rite	Yes	No	Top	PC = 19 PM = 107	No	Questionable, 2 *Bg* spores at edge of hole.

TABLE I (Continued)
SUMMARY OF EXPERIMENTAL RESULTS

Test Number	After Freeze Drying B. globigii (Bg) stored over:	∝ Source in Vibrator M* Chamber	∝ Source in Desic-cator	Position of Cell	Pressure C* Pressure M*	Vibrator On While Injecting Air	Results
10	Dri-rite	Yes	No	Top	PC = 58 PM = 160	No	Positive, hole plugged with spores, Cover slip (Top) - 0, Slide #1-2 Bg, Slide #2-3 Bg, Slide #3-4 Bg, Slide #4-3 Bg, Slide #5-2 Bg, Slide #6-0, End plate (Bot-tom)-45 Bg, End plate (Top)-0 Bg.

TABLE I (Continued)
SUMMARY OF EXPERIMENTAL RESULTS

Test Number	After Freeze Drying B. globigii (Bg) stored over:	∝ Source in Vibrator M* Chamber	∝ Source in Desic-cator	Position of Cell	Pressure C* Pressure M*	Vibrator On While Injecting Air	Results
11	Dri-rite	Yes	No	Bottom	PC = 58 PM = 112	No	Vibrator membrane fell into main chamber, test stopped.
12	Dri-rite	Yes	No	Bottom	PC = 53 PM = 95	No	Negative
13	Dri-rite	Yes	No	Top	PC = 52 PM = 550	No	Positive, hole plugged with Bg spores (Approximately 50 spores in hole).

TABLE I (Continued)
SUMMARY OF EXPERIMENTAL RESULTS

Test Number	After Freeze Drying B. globigii (Bg) stored over:	∝ Source in Vibrator M* Chamber	∝ Source in Desic-cator	Position of Cell	Pressure C* Pressure M*	Vibrator On While Injecting Air	Results
14	NaOH	No	Yes	Top	PC = 49	Yes	Positive, hole-33 Bg, cover slip 4 Bg, Slide #1-5 Bg, Slide #4-26 Bg, Slide #2-5 Bg, Slide #5-208 Bg (shot-gun pattern appearance) Slide #3-22 Bg, Slide #6-34 Bg.

TABLE I (Continued)
SUMMARY OF EXPERIMENTAL RESULTS

Test Number	After Freeze Drying B. globigii (Bg) stored over:	% Source in Vibrator M* Chamber	% Source in Desiccator	Position of Cell	Pressure C* Pressure M*	Vibrator On While Injecting Air	Results
15	NaOH	No	Yes	Top	PC = 45 PM = 140	Yes	Positive, hole - 14 Bg, cover slip-7 Bg, Slide #1-0 Bg, Slide #2-0 Bg, Slide #3-1 Bg, Slide #4-10 Bg, Slide #5-0 Bg, Slide #6-about 400 Bg (shotgun appearance)
16	NaOH	No	Yes	Top	PC = 310 PM = 10	Yes	Negative
17	NaOH	No	Yes	Top	PC = 52 PM = 90	Yes	Positive

TABLE I (Continued)
SUMMARY OF EXPERIMENTAL RESULTS

Test Number	After Freeze Drying B. globigii (Bg) stored over:	∝ Source in Vibrator M* Chamber	∝ Source in Desic-cator	Position of Cell	Pressure C* Pressure M*	Vibrator On While Injecting Air	Results
18	NaOH	No	Yes	Top	PC = 52 PM = 90	Yes	Positive, Bg found to have passed through hole.
19	New dispersion technique evaluation using injection principle; problem with leakage of injection device.						

TABLE II

BACILLUS GLOBIGII SPORE CLUMP SIZE DISTRIBUTION FOR LOW PRESSURE TESTS

Test Number 1

Slide No. and Position on Slide	Total No. of Spore Clumps	Number of Spore Clumps Larger than 1 MIL	% of Spore Clumps Larger than 1 MIL	Number of Spore Clumps Smaller than 1 MIL	% of Spore Clumps Smaller than 1 MIL
1 TOP HALF	139	15	10.8%	124	89.2%
1 BOTTOM HALF	170	42	24.7%	128	75.3%
2 TOP HALF	95	14	14.7%	81	85.3%
2 BOTTOM HALF	189	24	12.7%	165	87.3%
3 TOP HALF	18	8	44.3%	10	55.7%
3 BOTTOM HALF	18	8	44.3%	10	55.7%

TABLE II (Continued)

BACILLUS GLOBIGII SPORE CLUMP SIZE DISTRIBUTION FOR LOW PRESSURE TESTS

Test Number 2

Slide No. and Position on Slide	Total No. of Spore Clumps	Number of Spore Clumps Larger than 1 MIL	% of Spore Clumps Larger than 1 MIL	Number of Spore Clumps Smaller than 1 MIL	% of Spore Clumps Smaller than 1 MIL
1 TOP HALF	305	32	10.5%	273	89.5%
1 BOTTOM HALF	248	35	14.1%	213	85.9%
2 TOP HALF	206	11	5.4%	195	94.6%
2 BOTTOM HALF	158	12	7.6%	146	92.4%
3 TOP HALF	214	26	12.1%	188	87.9%
3 BOTTOM HALF	95	16	16.8%	79	83.2%

TABLE II (Continued)

BACILLUS GLOBIGII SPORE CLUMP SIZE DISTRIBUTION FOR LOW PRESSURE TESTS

Test Number 3

Slide No. and Position on Slide	Total No. of Spore Clumps	Number of Spore Clumps Larger than 1 MIL	% of Spore Clumps Larger than 1 MIL	Number of Spore Clumps Smaller than 1 MIL	% of Spore Clumps Smaller than 1 MIL
1 TOP HALF	92	12	13.0%	80	87.0%
1 BOTTOM HALF	104	15	14.4%	89	85.6%
2 TOP HALF	80	23	28.7%	57	71.3%
2 BOTTOM HALF	116	24	20.6%	92	79.4%
3 TOP HALF	151	11	7.3%	140	92.7%
3 BOTTOM HALF	76	16	21.0%	60	79.0%

TABLE II (Continued)

BACILLUS GLOBIGII SPORE CLUMP SIZE DISTRIBUTION FOR LOW PRESSURE TESTS

Test Number 4

Slide No. and Position on Slide	Total No. of Spore Clumps	Number of Spore Clumps Larger than 1 MIL	% of Spore Clumps Larger than 1 MIL	Number of Spore Clumps Smaller than 1 MIL	% of Spore Clumps Smaller than 1 MIL
1 TOP HALF	101	15	14.8%	86	85.2%
1 BOTTOM HALF	116	22	19.0%	94	91.0%
2 TOP HALF	90	20	22.2%	70	77.8%
2 BOTTOM HALF	100	20	20.0%	80	80.0%
3 TOP HALF	120	20	16.7%	100	83.3%
3 BOTTOM HALF	100	26	26.0%	74	74.0%

TABLE III

PROPOSED TEST SEQUENCES BASED UPON A THEORETICAL VIOLATION OCCURRING AT A ΔP OF FOUR INCHES OF WATER AFTER ONE HOUR TEST

Durations of Test in Hours

ΔP, pressure differential value for test chamber vs. main chamber (forward flow), Inches H_2O	1/2 hr.	1 hr.	2 hrs.	4 hrs.	48 hrs.
4		P_N^1			
3		P_N^2			
2		P_N^3			
1		P_N^4			S^{10}
1/2		P_N^5	P^{13B}	$P^{12B}+S^{11B}$	S^9
1/4	P^{11A}	P_Y^6	P^{13C}	$P^{12C}+S^{11C}$	S^8
+	S*	P^7			
Reverse flow (proof test) main chamber at higher pressure than test chamber P, Inches H_2O					
4	P^o (to be performed before forward flow tests)				

P = use of small Petri dish (approx. 50 mm. diameter) over orifice in test chamber, this is to concentrate possible violating Bg spores into a small area.

S = use of glass slide assembly in test chamber to study distribution of violating Bg spores.

The superscript number above the P or S (P^1, P^2, P^3, etc.) indicates the order in which the tests are to be performed. The letter beside the superscript indicates series of the test.

Subscript N (P_N^5) indicates no violation occurred; subscript Y (P_Y^{6A}) indicates violations occurred.

If P_Y^6 has a large amount of violation (Bg) do "A" series of tests; if S^9 has violations do "B" series of tests; if P_Y^6 has limited amount of violations and S^9 no violations do "C" series of tests.

of the orifice by B. globigii spores could be seen in the Petri dish. The results of these tests are presented in Table IV. Photographs of these test results are presented in Appendix B.

Calibration of Challenge. - The procedure for the violation tests is detailed on page 15. It is indicated there that the challenge organisms were introduced by injection through a hypodermic needle. A sequence of auxiliary tests described on page 20 was conducted to measure the variation of the aerosol density distribution with time. The results of this experimentation are shown in Figure 19 in Appendix A. Counts were made to determine particulate count per unit volume of the aerosol sampled after time periods of one minute, three minutes, six minutes, 12 minutes, and 20 minutes. This was repeated for heights of 0, 1/2 inch, 1 inch, 1 1/2 inches above the main chamber floor. It is evident that the concentration of spores, n(t), is a highly reproducible function of time and is relatively independent of height.

In Appendix A it is shown that, for this experimentally obtained n(t), the effective challenge over a time period t, c(t), can be represented by

$$c(t) = n(0)\, t_c\, f(t), \tag{2}$$

where:

$$n(0)\, t_c = \int_0^\infty n(t)\, dt$$

$$= 1.635 \times 10^5 \text{ spore-minutes/cm}^3,$$

i.e., the challenge represented by a constant concentration of 1.635 x 10 spores/cm maintained for one minute, and f(t) is the fraction of the original concentration which has precipitated out of the air at time t. In particular,

$$f(0) = 0 \ ;$$

$$f(\infty) = 1 \ . \tag{3}$$

Evaluation of f(t) for arbitrary t is discussed in Appendix A.

Violation tests. - The results of the orifice violation tests for a 0.006 inch hole diameter are given in Table V. The results for 0.010 and 0.020 inch holes are given in Tables VI and VII, respectively. The significant data to be noted for each run are: (1) hole size, (2) pressure differential, (3) effective challenge, and (4) approximate number of violations observed. The

TABLE IV

RESULTS OBTAINED PROOF-TESTING THE EXPERIMENTAL SYSTEM UTILIZING A B. GLOBIGII CHALLENGE AND 6 MIL, 10 MIL, AND 20 MIL ORIFICES

Proof Test Number	Orifice Size In MILs	Pressure Differential* ΔP	Number of Bg Spores Per Ml of Air ≠	Duration of Test in Minutes	Approx. Number of Bg Spores Violating Orifice	Remarks
1	6	4 inches H_2O	3×10^4	8	10^7	⊕
2	10	4 inches H_2O	3×10^4	8	10^8	⊕
3	20	4 inches H_2O	3×10^4	8	10^9	⊕

* Main Chamber at higher pressure than the test chamber.

≠ Estimated number of B. globigii spores per Ml of air in the Main Chamber using a membrane filter technique for air sampling.

⊕ Tests stopped after eight minutes, violation of orifice was apparent by visual observation of Petri dish in the test cell.

TABLE V

RESULTS OBTAINED PERFORMING EXPERIMENTAL TRIALS WITH A 6 MIL ORIFICE AT VARIOUS PRESSURE DIFFERENTIALS AND FOR DIFFERENT PERIODS OF TIME

Experimental Test Number	Orifice Size In MILs	Pressure Differential ΔP	Duration of Test In Hours	Challenge In Spore-Minutes Per Ml of Air	Approximate Number of Bg. Spores Violating Orifice	
					Experimental	Calculated From Model
1	6	4 in. water	1	9.1×10^5	0	$<10^{-10}$
2	6	3 in. water	1	1.1×10^6	0	$<10^{-10}$
3	6	2 in. water	1	9.5×10^5	0	3.8×10^{-8}
4	6	1 in. water	1	8.8×10^5	0	0.015
5	6	1/2 in. water	1	9.6×10^5	0	11
6	6	1/4 in. water	1	9.6×10^5	302	296
7	6	Ambient Pressure	1	1.1×10^6	8,900	8,805
8	6	1/2 in. water	48			
9	6	1/4 in. water	2	1.7×10^6	250	547
10	6	1/4 in. water	1	9.5×10^5	400	291
11	6	1/4 in. water	5	2.9×10^6	13	903

TABLE VI

RESULTS OBTAINED PERFORMING EXPERIMENTAL TRIALS WITH A 10 MIL ORIFICE AT VARIOUS PRESSURE DIFFERENTIALS AND FOR DIFFERENT PERIODS OF TIME

Experimental Test Number	Orifice Size In MILs	Pressure Differential ΔP	Duration of Test In Hours	Challenge In Spore-Minutes Per Ml of Air	Approximate Number of Bg. Spores Violating Orifice	
					Experimental	Calculated From Model
1	10	4 in. water	1	1.1×10^6	0	$<10^{-10}$
2	10	3 in. water	1	8.8×10^5	0	$< 10^{-10}$
3	10	2 in. water	1	7.8×10^5	0	8.8×10^{-8}
4	10	1 in. water	1	9.5×10^5	0	0.047
5	10	1/2 in. water	1	9.6×10^5	0	31
6	10	1/4 in. water	1	9.3×10^5	TNTC*	795
7	10	Ambient Pressure	1	9.1×10^5	TNTC*	2.0×10^4
8	10	1/2 in. water	48		0	
9	10	1/4 in. water	2	1.7×10^6	TNTC*	1534

*Too numerous to count.

TABLE VII

RESULTS OBTAINED PERFORMING EXPERIMENTAL TRIALS WITH A 20 MIL ORIFICE AT VARIOUS PRESSURE DIFFERENTIALS AND FOR DIFFERENT PERIODS OF TIME

Experimental Test Number	Orifice Size In MILs	Pressure Differential ΔP	Duration of Test In Hours	Challenge In Spore-Minutes Per Ml of Air	Approximate Number of Bg. Spores Violating Orifice	
					Experimental	Calculated From Model
1	20	4 in. water	1	9.5×10^5	0	$<10^{-10}$
2	20	3 in. water	1	9.5×10^5	0	$<10^{-10}$
3	20	2 in. water	1	9.5×10^5	0	1.2×10^{-7}
4	20	1 in. water	1	9.6×10^5	0	5.7×10^{-2}
5	20	1/2 in. water	1	9.6×10^5	Plug	38
6	20	1/4 in. water	1	9.6×10^5	TNTC *	987
7	20	1/4 in. water	1/2	4.7×10^5	TNTC *	485
8	20	1/2 in. water	4	2.4×10^6	TNTC *	97
9	20	1 in. water	4	2.1×10^6	Plug	0.13
10	20	Ambient	1	9.3×10^5	TNTC *	2.5×10^4

*Too numerous to count.

challenge quoted for each run is the result of a sequence of injections spaced about 10 minutes apart. This is calculated by adding the individual challenge values for the test duration times after each injection:

$$c(t) = 1.64 \times 10^5 \sum_{i=1}^{N} f(t_i), \qquad (4)$$

where $f(t_i)$ is the fractional concentration function mentioned above evaluated for the ith injection. The data can be fitted to a simple model developed in Appendix A. This model gives the number of violations expected as:

$$N(t) = 2.2 \times 10^{-4} D^2 c(t) e^{-13 \Delta P}, \qquad (5)$$

where D is the diameter of the orifice in MILS, ΔP is the pressure differential in inches of water and c(t) is the challenge as given by Equation 4. The probable number of violations based on this model are given, for comparison, in Tables V-VII. In most instances, the observed data took the appearance of a compact clustering of spores found on a spot directly beneath the test orifice. As a consequence, the only objective evaluation of spore numbers possible in a number of instances was TNTC (too numerous to count). However, subjective judgment of the numbers of spores in these clusters generally was consistent with the model predictions.

Particle size distribution for atmospheric tests. - For the atmospheric tests, particles were counted and sized only when violation occurred through the hole on the Petri dish. Figures 10 to 13 show the distribution of the particle counts and sizes. Counts were recorded for the 10 MIL hole at a ΔP of 1/4 inch water for one hour and two hours. Counts were also made for the 20 MIL hole at a ΔP of 1/4 inch water for 1/2 hour and one hour.

Comparison of Desiccated *B. globigii* Spores With Freeze-Dried *B. globigii* Spores

After standardization, the slides were placed on the stage of the microscope and irradiated at 425 mμ. The level of fluorescence was determined for an individual microorganism in the scanning area (using the micromechanical stage control) and reading the resultant values from the photomultiplier meter. The photomultiplier was zeroed to the background light level before readings were taken. The results, as shown in Figure 14, were recorded and graphed as the frequency of occurrence versus the fluorescent light level produced when excited at a specific wavelength (425 mμ).

FIGURE 10
PARTICLE DISTRIBUTION - 10 MIL HOLE

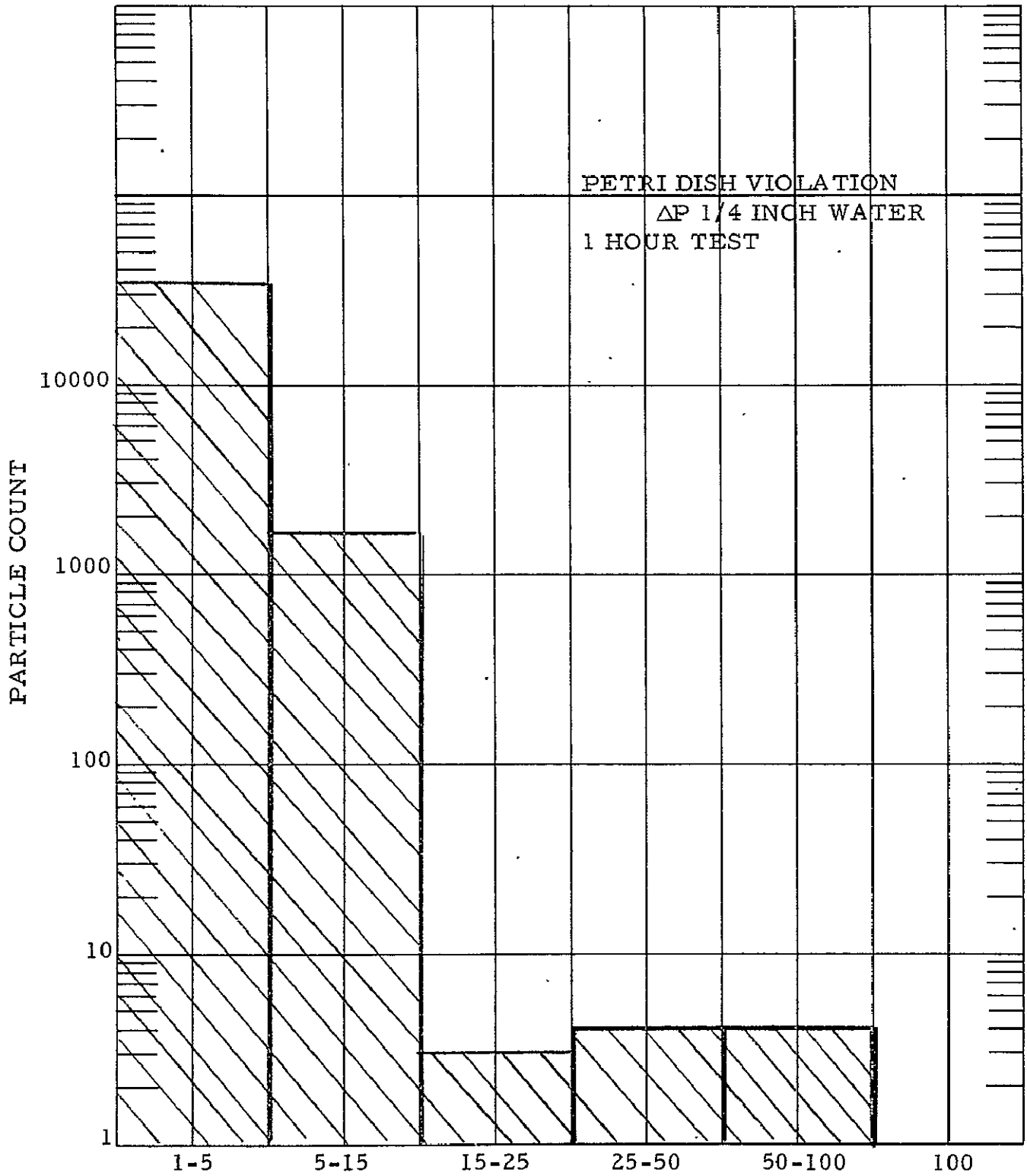


FIGURE 11

PARTICLE DISTRIBUTION - 10 MIL HOLE

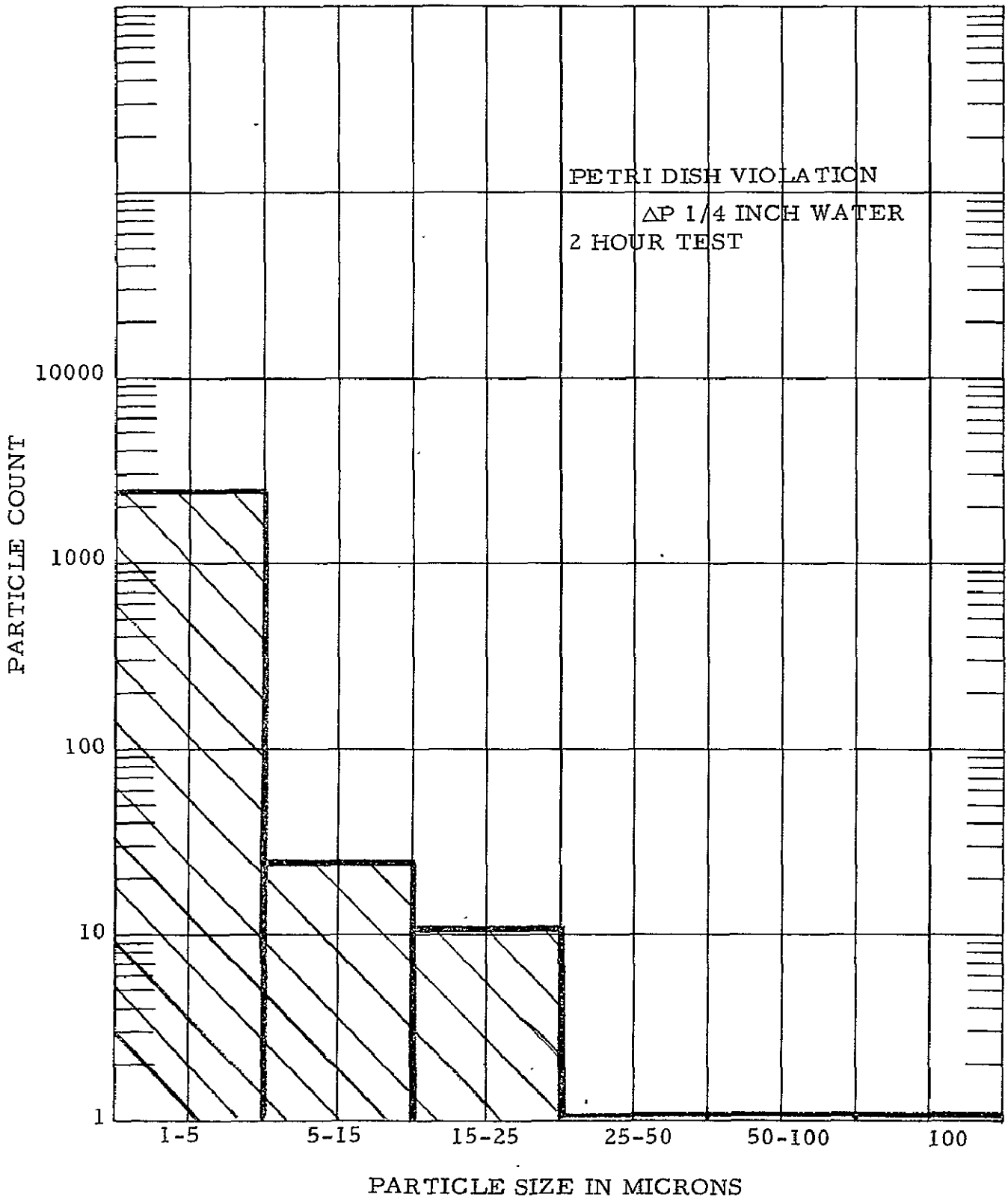


FIGURE 12

PARTICLE DISTRIBUTION - 20 MIL HOLE

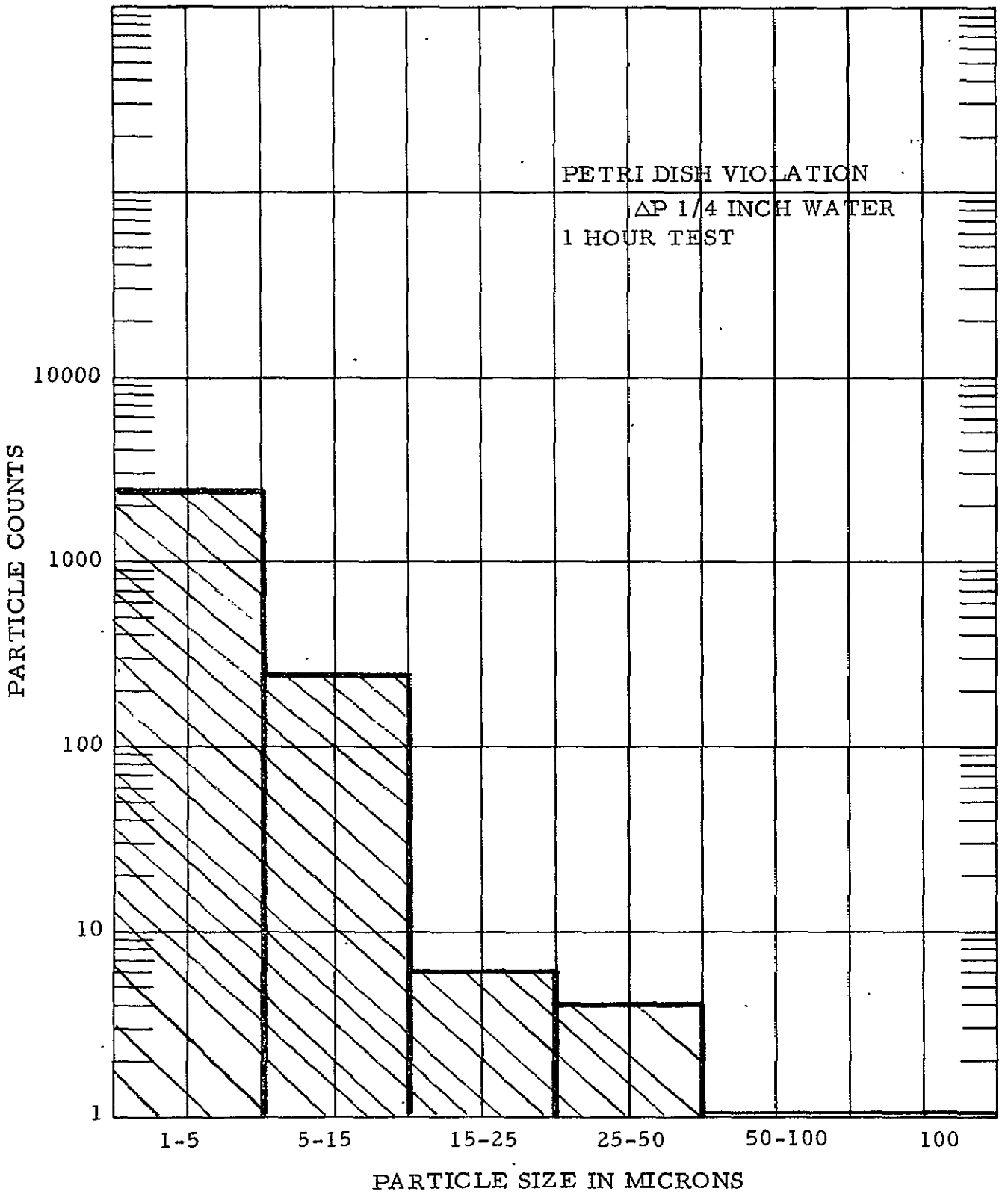


FIGURE 13
PARTICLE DISTRIBUTION - 20 MIL HOLE

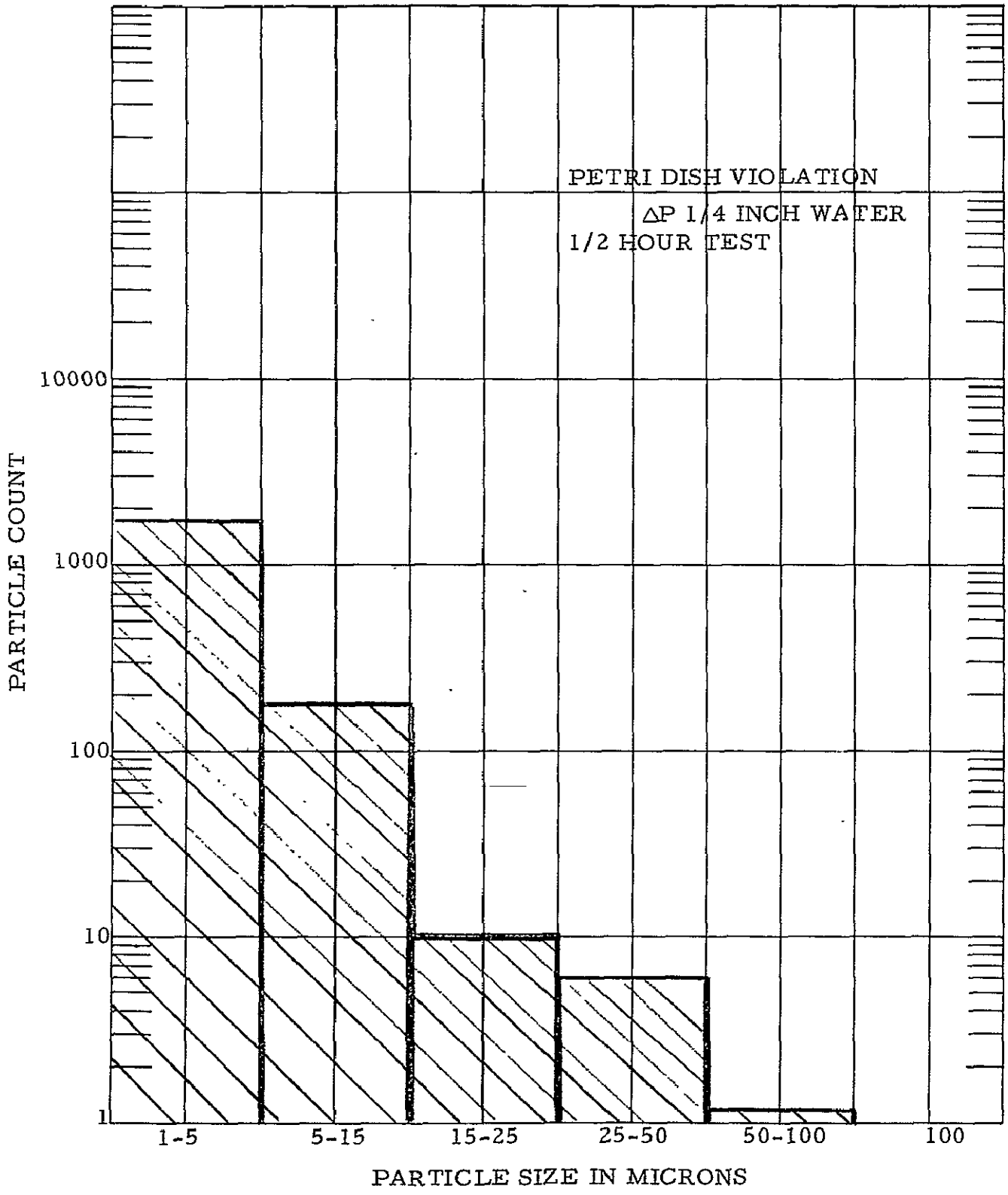
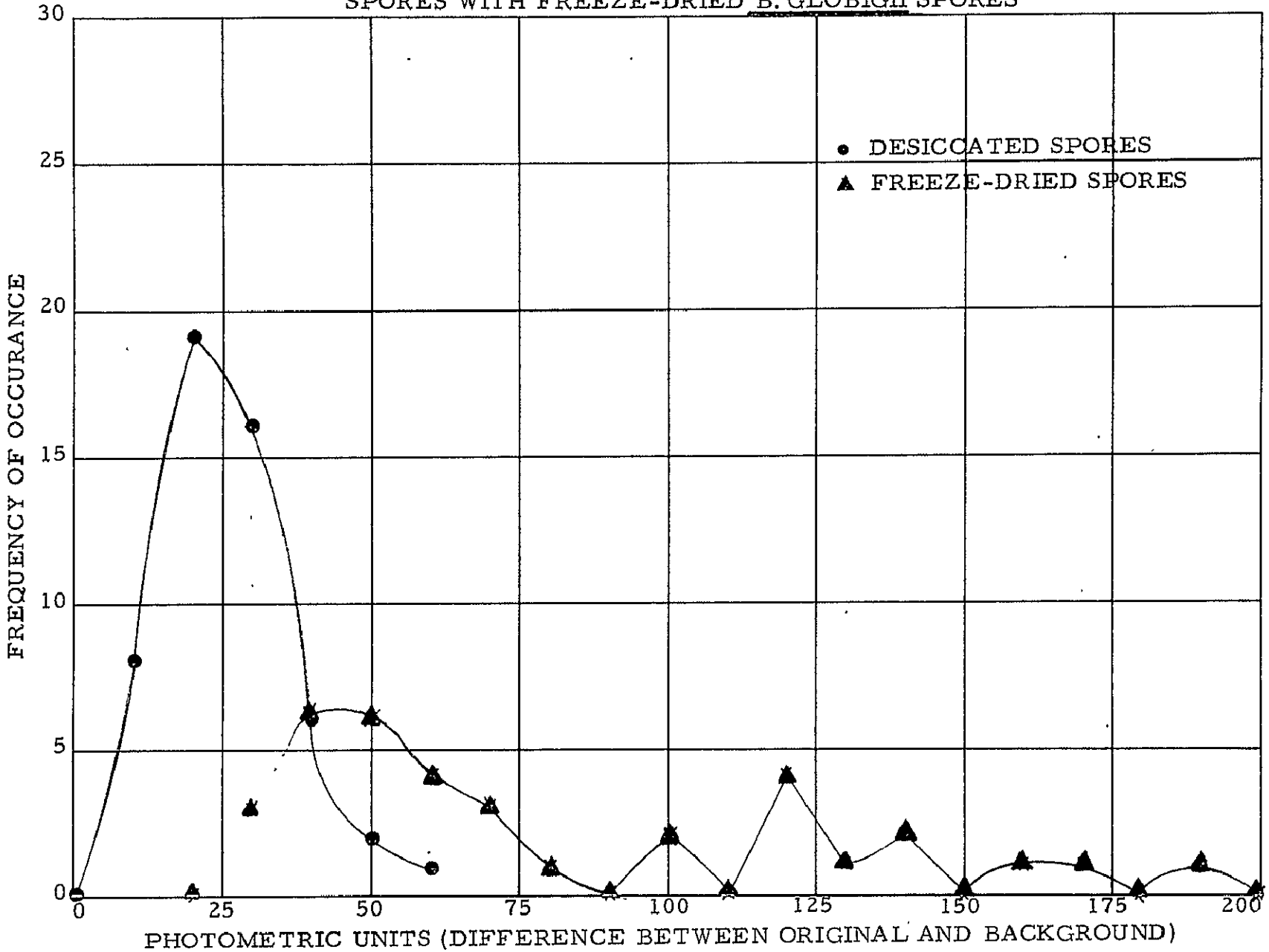


FIGURE 14
COMPARISON OF DESICCATED B. GLOGIGII
SPORES WITH FREEZE-DRIED B. GLOBIGII SPORES



CONCLUSIONS

The results of tests performed in the low pressure regime are inconclusive. However, in the tests performed the pressure gradient was pre-dominantly applied in the direction that would aid violation of the test hole. Although no statistical statement can be confidently made because of the lack of consistent data the successful violation in this regime for a bacterial challenge approaching a hole in a bio-barrier at low velocity is quite small. Moreover, as the size hole (0.001 inch-diameter) for which data has been gathered is substantially larger than would be allowed in a bio-barrier before a vehicle launch, the possibility of recontamination seems even more remote in this regime. The remaining problem that has not been addressed in this work is concerned with the possibility of violation when combined force fields are applied to holes in a bio-barrier with holes both larger (from micrometeorite puncture) and smaller (manufacturing defects) than used in the low pressure tests.

The test technique problems encountered in the low pressure tests will be of great assistance to future investigators in that sufficiently more sophisticated bacterial dispersion devices must be employed than those allowed within the scope and funding of this program.

In the atmospheric tests, it has been the consistent result that orifice violations do not occur at impeding differential pressures greater than approximately 0.5 inches of water. Experimentally, we have established this result only for orifice diameters in the range of six to 20 MILS, for challenges of about 10^6 spore-minutes/ml magnitude, and particle sizes in the range from one to $\sim 50\ \mu$.

A simple model whereby estimates may be obtained of probable numbers of violations for arbitrary orifice size, pressure differential, and challenge has been developed. When fitted to the experimental results the model can be summarized by the relation:

$$N(t) = 2.2 \times 10^{-4}\, D^2\, c(t)\, e^{-13\,\Delta P}, \qquad (5)$$

where D is the orifice diameter in MILS, c(t) is the challenge as defined on page 40, and ΔP is the pressure differential in inches of water. The dependence of this relation on a factor D^2 is a consequence of assumptions of laminar flow and of an orifice thickness negligible compared to its diameter. The assumption of laminar flow is a matter of convenience, since it would be extremely difficult to treat turbulent flow in the case of realistic orifice profiles. Nonetheless, it can be argued that near the

threshold of orifice violation the dominant effect may be turbulence near the orifice edge. In this case, a dependence on orifice circumference rather than area might be expected (i.e., a factor D instead of D^2). The data given in this report can be fitted by either D dependence. For orifice diameters smaller than 6 MILS a linear dependence on D gives the worst case (i.e., predicts the most violations). For this case, the fitted equation is

$$N(t) = 1.3 \times 10^{-3} D\, c(t)\, e^{-13 \Delta P} \qquad (6)$$

This equation may be used for effective $D < 6$ MILS (orifice diameter diminished by the diameter of the challenge particle) to obtain upper limits of the number N(t).

A further comment on the assumption of laminar flow can be made. A series of photographs were made of smoke being blown through the 0.020 inch diameter orifice used in the violation tests. By using the smoke the air flow patterns for different pressure differentials were made visible. These are shown in Figures 15-18. It is apparent that for low differential pressures the laminar stream extends for several inches. When the pressure difference is as large as four inches of water turbulence is still some distance (about 1/8 inch) from the orifice.

Langley Research Center,
National Aeronautics and Space Agency,
Langley Station,
Hampton, Virginia, 23365, 6 November 1968.

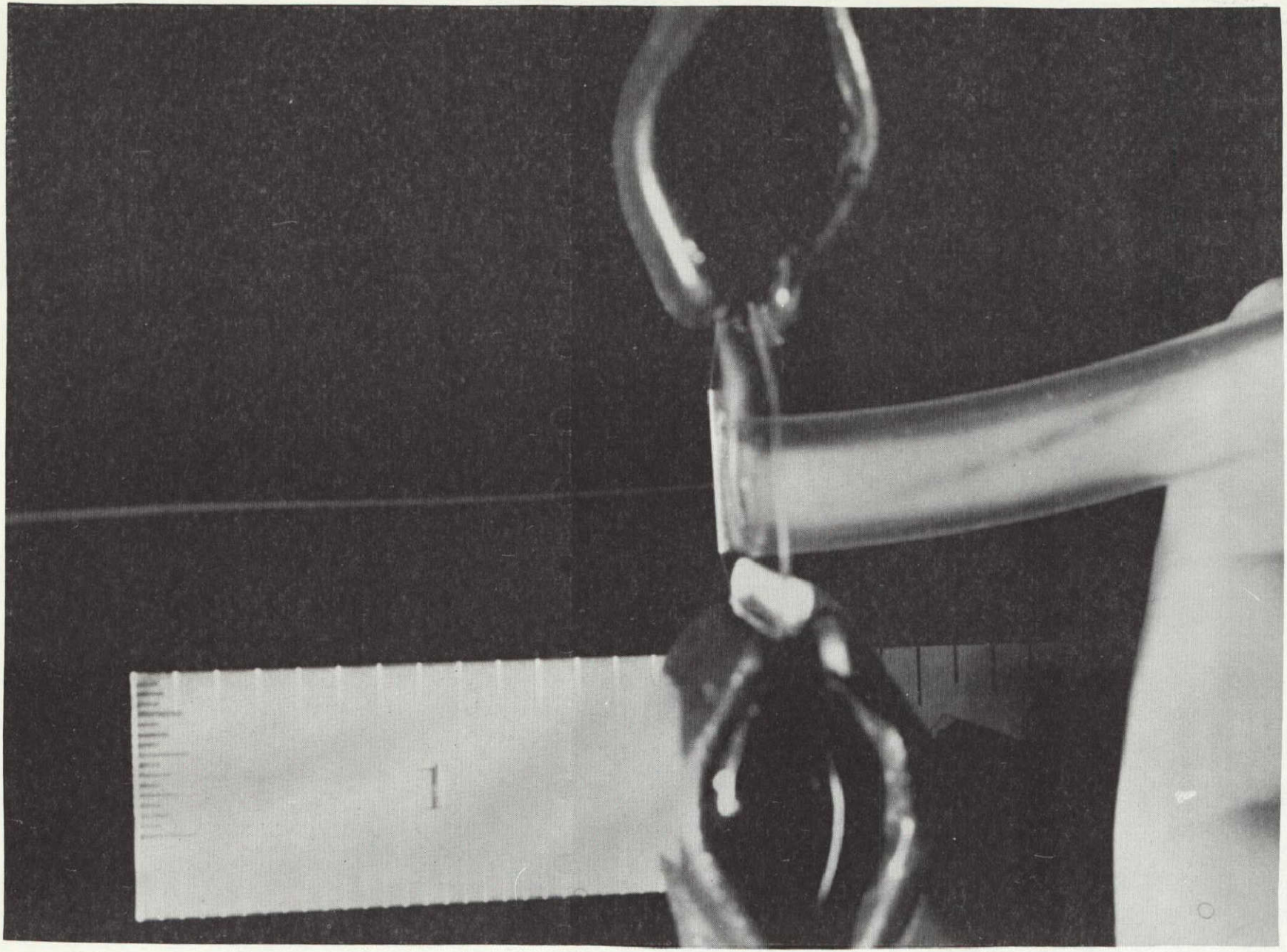


FIGURE 15. - DEMONSTRATION OF LAMINAR FLOW

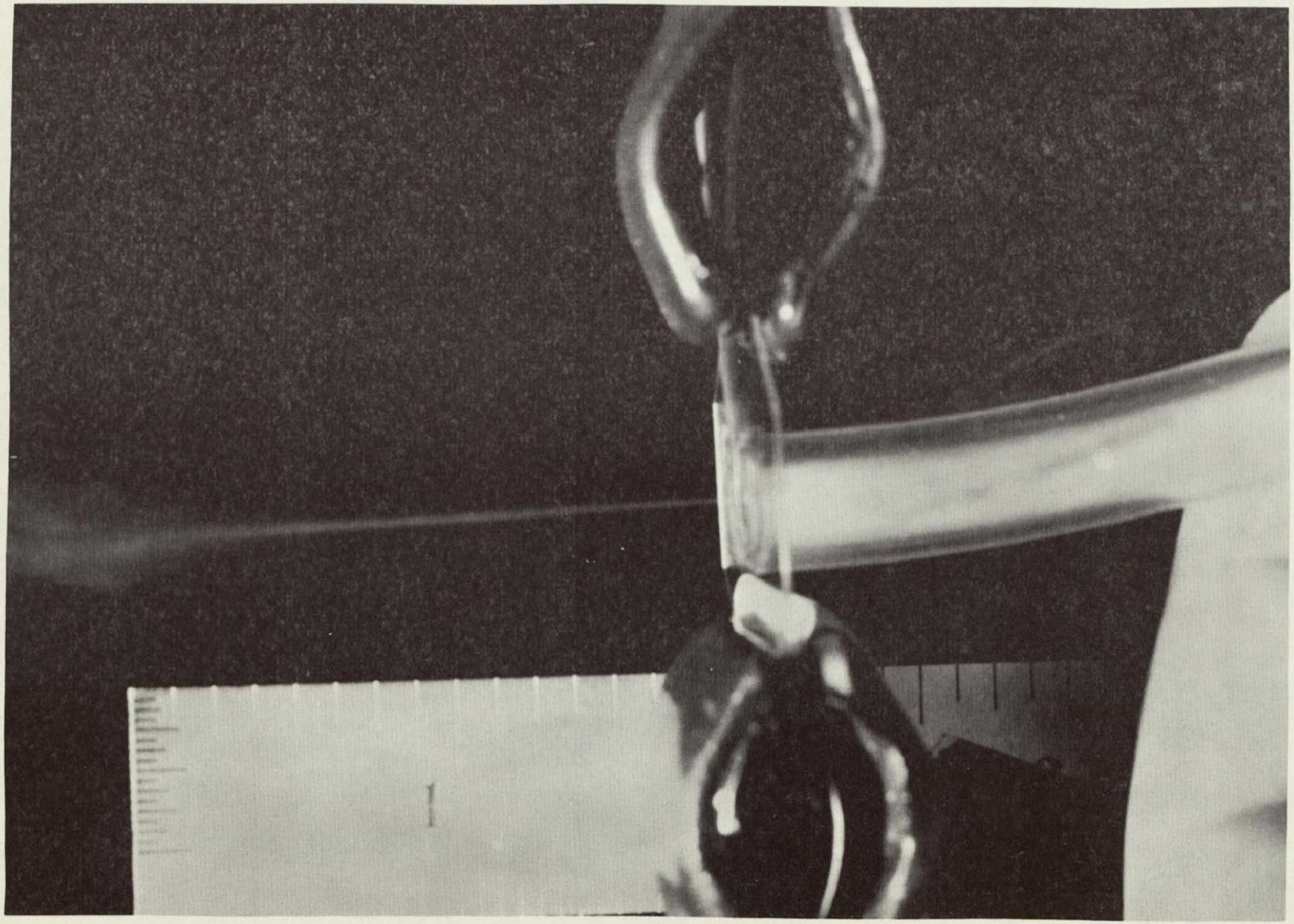


FIGURE 16. - DEMONSTRATION OF LAMINAR FLOW
THROUGH 20 MIL ORIFICE PRESSURE ~1 INCH WATER

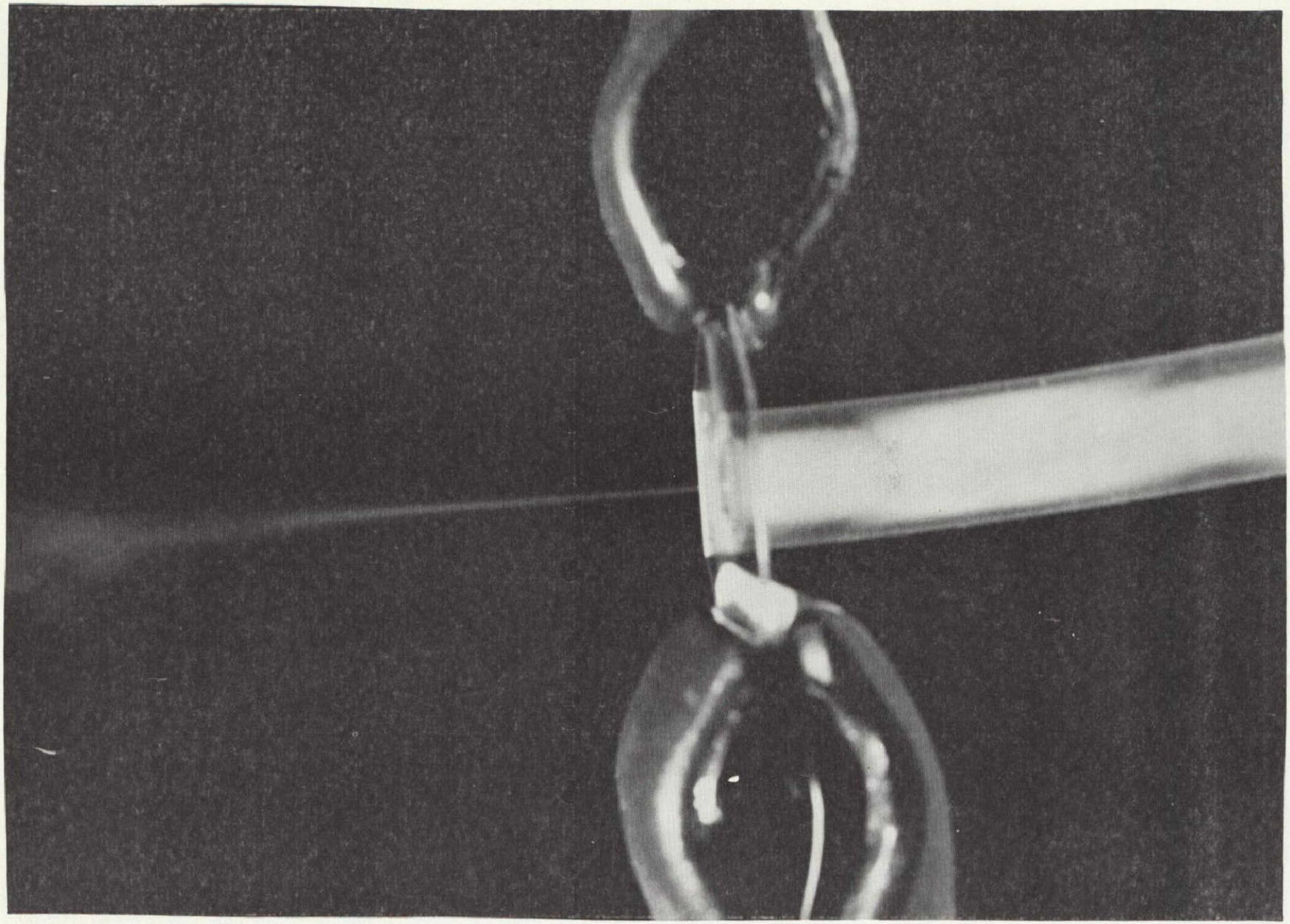


FIGURE 17. - DEMONSTRATION OF LAMINAR FLOW
THROUGH 20 MIL ORIFICE PRESSURE ~2 INCHES WATER

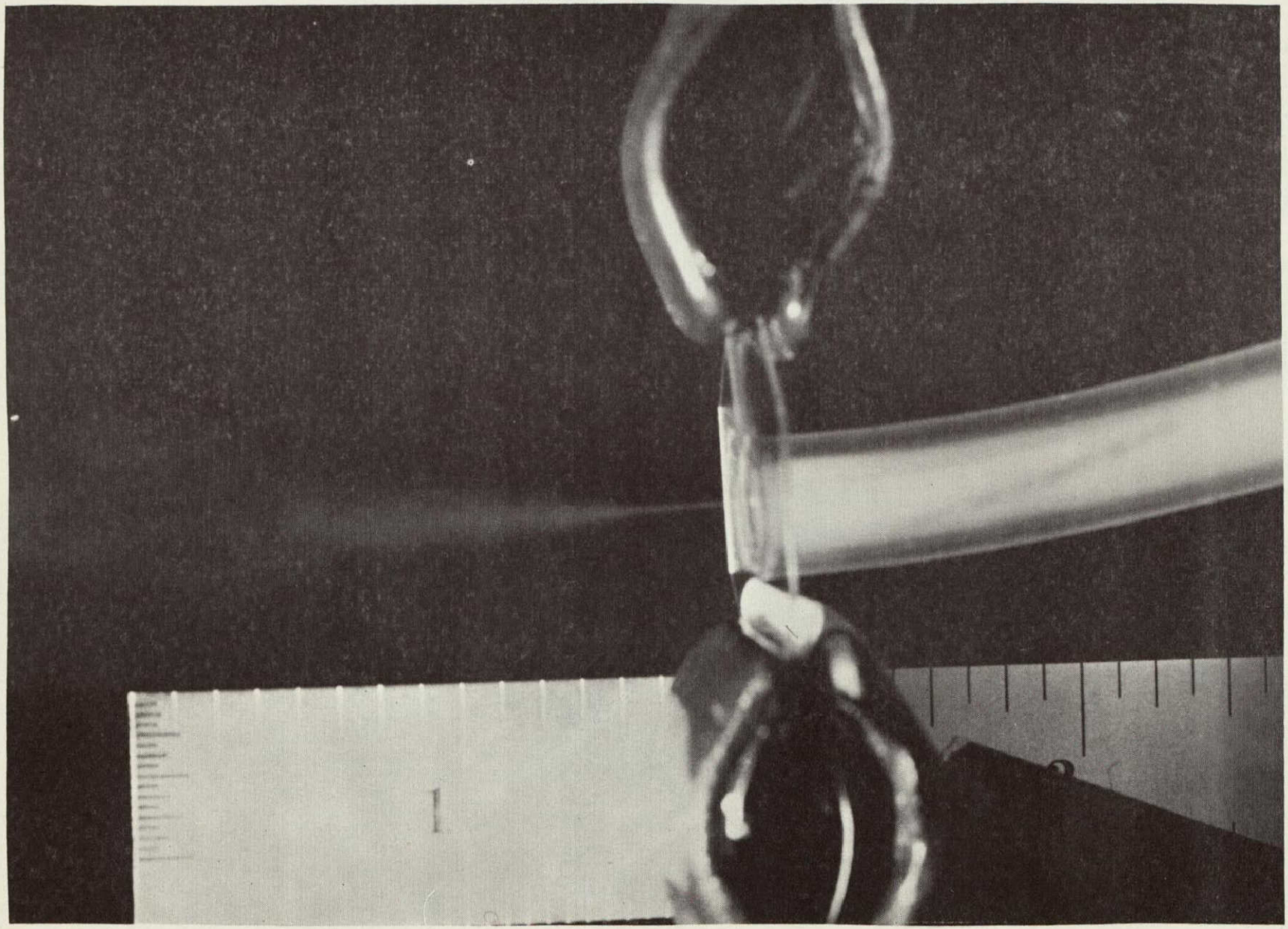


FIGURE 18. - DEMONSTRATION OF LAMINAR FLOW
THROUGH 20 MIL ORIFICE PRESSURE ~4 INCHES WATER

APPENDIX A

SIMPLE MODEL OF ORIFICE VIOLATION AT NEARLY ATMOSPHERIC PRESSURES

Assume two chambers separated by a very thin diaphragm pierced by a single circular hole of area A. Let the upper chamber contain air at atmospheric pressure P_o, and let the lower chamber contain air at a slight overpressure $P_o + \Delta P$ ($\Delta P << P_o$). There is a steady flow of air through the orifice from lower to upper chamber with a flow velocity V_f proportional to ΔP, namely

$$V_f = k_f \Delta P$$

Near the orifice, the flow is perpendicular to the diaphragm. This flow pattern quickly diverges, of course, and loses identity a few hole-diameters away.

Consider a suspension of particles (in this application, spores) in the upper chamber. For this analysis we will consider that the particles fall toward the diaphragm because of gravity. There are other possible forces, such as electrostatic attraction, or local winds (in this case, a fan). The particle diameters lie in the range 10^{-4} to 10^{-2} cm, with specific gravity of about unity. The limiting velocities from viscous drag against a gravitational force range from 10^{-2} to 10^{2} cm/sec. The effect of the air flow through the orifice is equivalent superimposing an upward velocity on the particles trying to flow through the hole. This can be looked at also as additional upward drag force on the particle. Average limiting velocities for particles under the influence of other forces in general are different, but the qualitative physics of the following analyses are similar.

The rate at which particles escape through the orifice from the upper to lower chamber (against a pressure ΔP) is:

$$(1) \qquad \frac{dN_v}{dt} = K' A_{eff}\ n(t) \exp(-b\Delta P)$$

where

N_v = number of particles violating orifice
A_{eff} = effective orifice area

$\approx$ area of orifice - projected area of particles

$n(t)$ = concentration of particles over hole at time t (particles/cm^3)

ΔP = pressure difference at orifice

b = constant (to be determined)

K' = proportionality factor (to be determined)

Equation (1) is a simple physically reasonable expression which correlates the variables in the problem and allows an extrapolation of the data to long times and low concentrations. Much more sophisticated models could be constructed, of course, but this is not justified without a considerably more detailed experiment.

In equation (1) the linear dependence in effective orifice area and concentration of particles above the orifice are essentially intuitive, and are probably good assumptions. The analytical dependence on pressure is chosen for the following reason: At very small pressure difference,

(2) $$e^{-b\Delta P} \approx 1 - b\Delta P$$

The approximate form of (2) is just that expected on the single model that the limiting velocity under ambient conditions ($\Delta P = 0$) is reduced by an air flow velocity (which is proportional to ΔP). However, the exponential form, instead of cutting of abruptly at a finite pressure ($\Delta P = b^{-1}$), tails off to small values at large pressures. This we feel more realistically represents the small but finite probability of a particle violating the orifice against a ΔP which would in one-dimensional flow theoretically stop the particle.

The total number of particles violating the orifice in time t is found by integrating equation (1).

(3) $$N(t) = K A_{eff} \exp(-b\Delta P) f(t)$$

where

(4) $$f(t) = \frac{\int_0^t n(t')\,dt'}{\int_0^\infty n(t')dt'}$$

and

(5) $$K = K' \int_0^\infty n(t')\,dt'$$

The new proportionality parameter K is constant only if the total number of particles, and their fallout rate, is reproducible in the particular experiments performed. In the case of a constant concentration, i.e., $n(t) = \bar{n}$, then the appropriate relation is:

$$N(t) = K A_{eff} \exp(-b\Delta P) \bar{n} t \tag{6}$$

Equation (6) is the appropriate form for a MAST-oriented problem, where the concentration of organisms is small but constant.

An independent experiment was performed to determine the form of n(t). The procedure was similar to that carried out on the primary tests. The results are shown on Figure 19. The concentration time history appears to be unexpectedly reproducible to better than 30 percent. The data can be fit to a form

$$\begin{aligned} n(t) &= 50,000 \exp(-t/2.365); \; 5 \leq 3 \text{ min.} \\ &= 24,000 \exp(-t/5.6); \; t \geq 3 \text{ min.} \end{aligned} \tag{7}$$

Equation 7 can be integrated to give

$$\begin{aligned} f(t) &= 0.724 \left[1-\exp(-t/2.365)\right] \; ; \; t \leq 3 \text{ min.} \\ &= 0.52 + 0.48 \left[1-\exp(-\left[t-3\right] \; 15.6)\right] \; ; \; t \geq 3 \text{ min.} \end{aligned} \tag{8}$$

$$\int_0^\infty n(t)\, dt = 1.635 \times 10^5$$

Figure 20 gives a plot of f(t).

FIGURE 19

DECREASE OF B. GLOBIGII SPORE CONCENTRATION AT HEIGHTS OF 0, 1/2", 1" AND 1 1/2" FOR 20 MINUTES

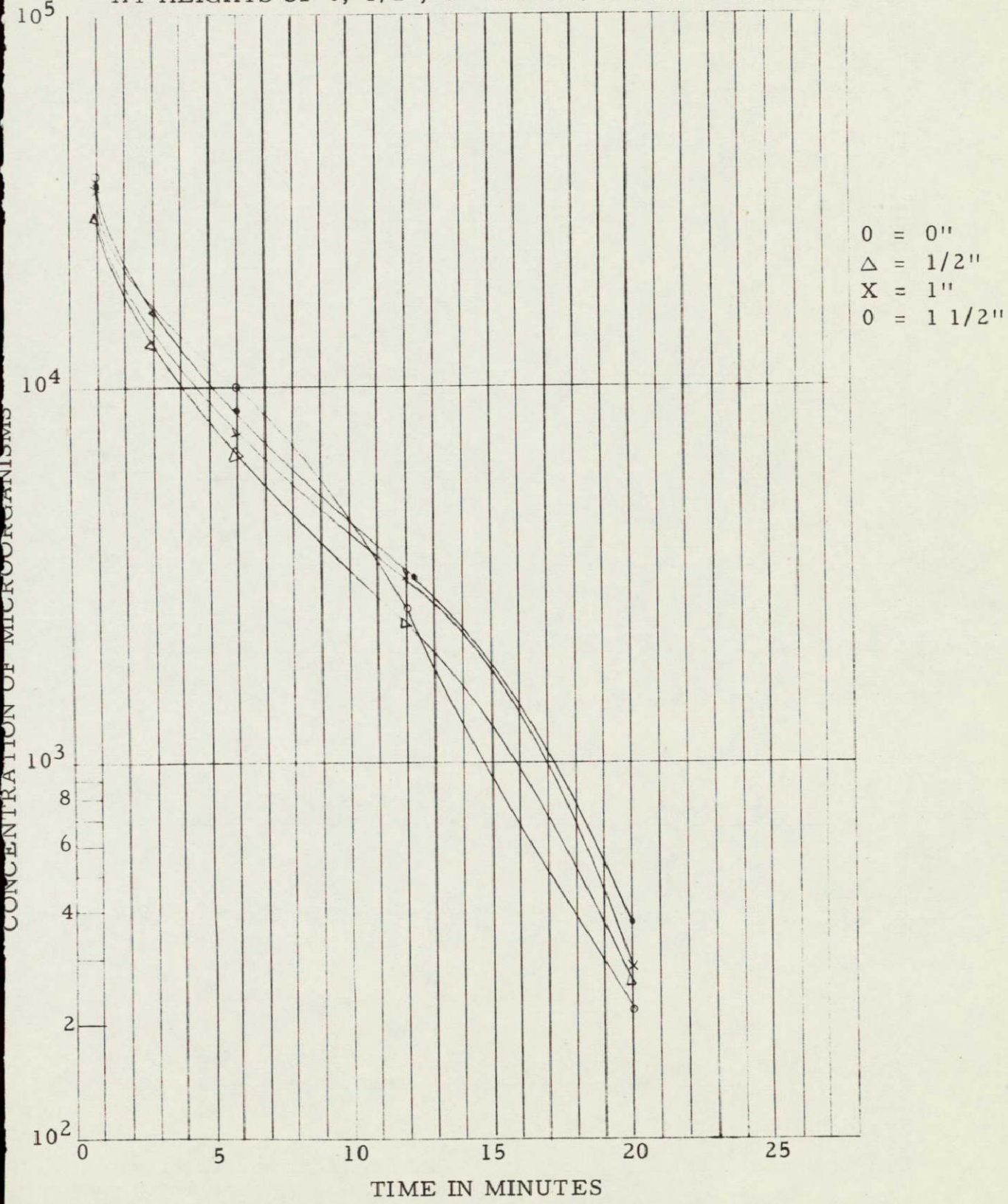
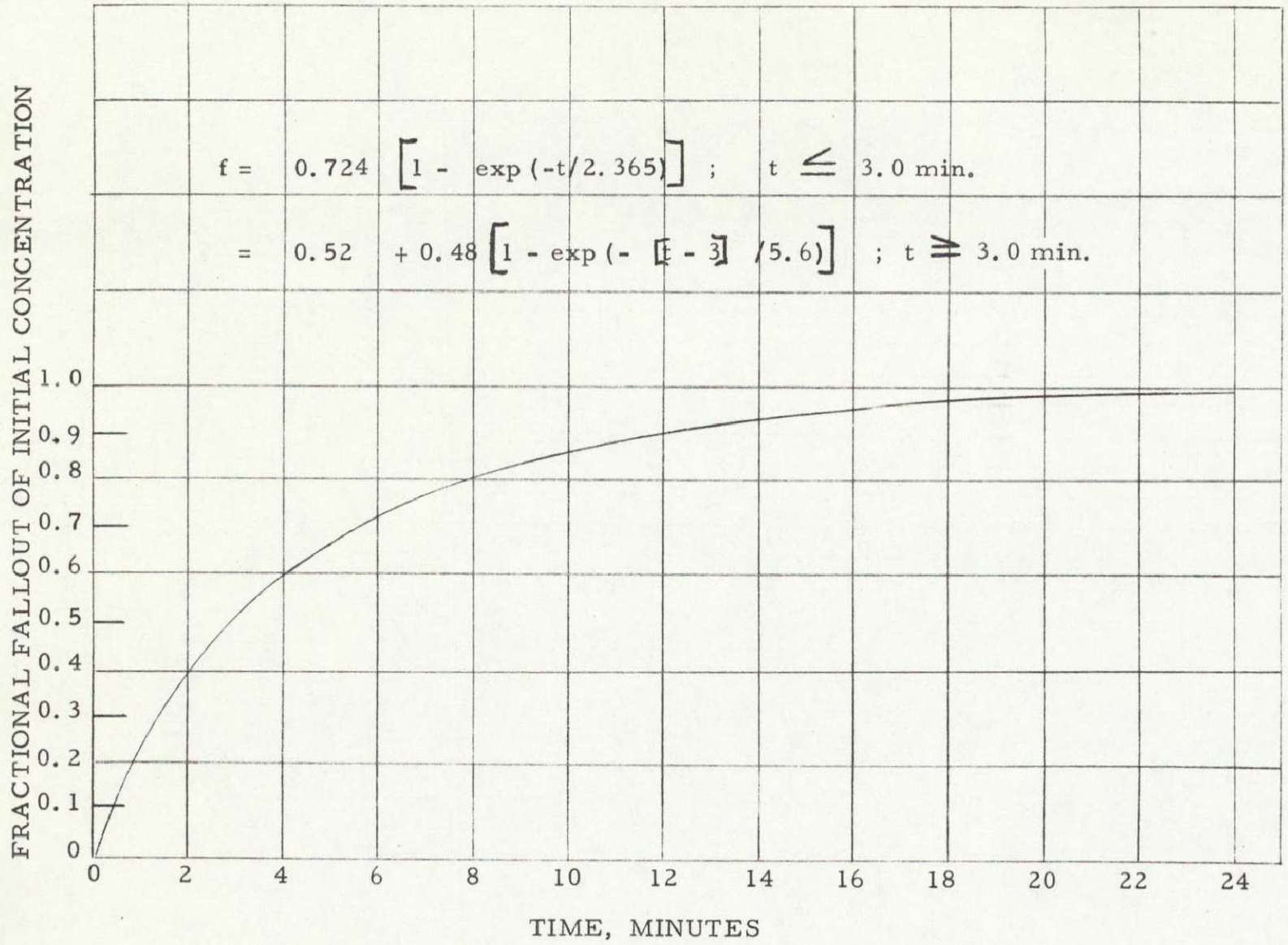


FIGURE 20

PLOT OF f(t)



APPENDIX B

PHOTOGRAPHS OF ORIFICE VIOLATION TESTS

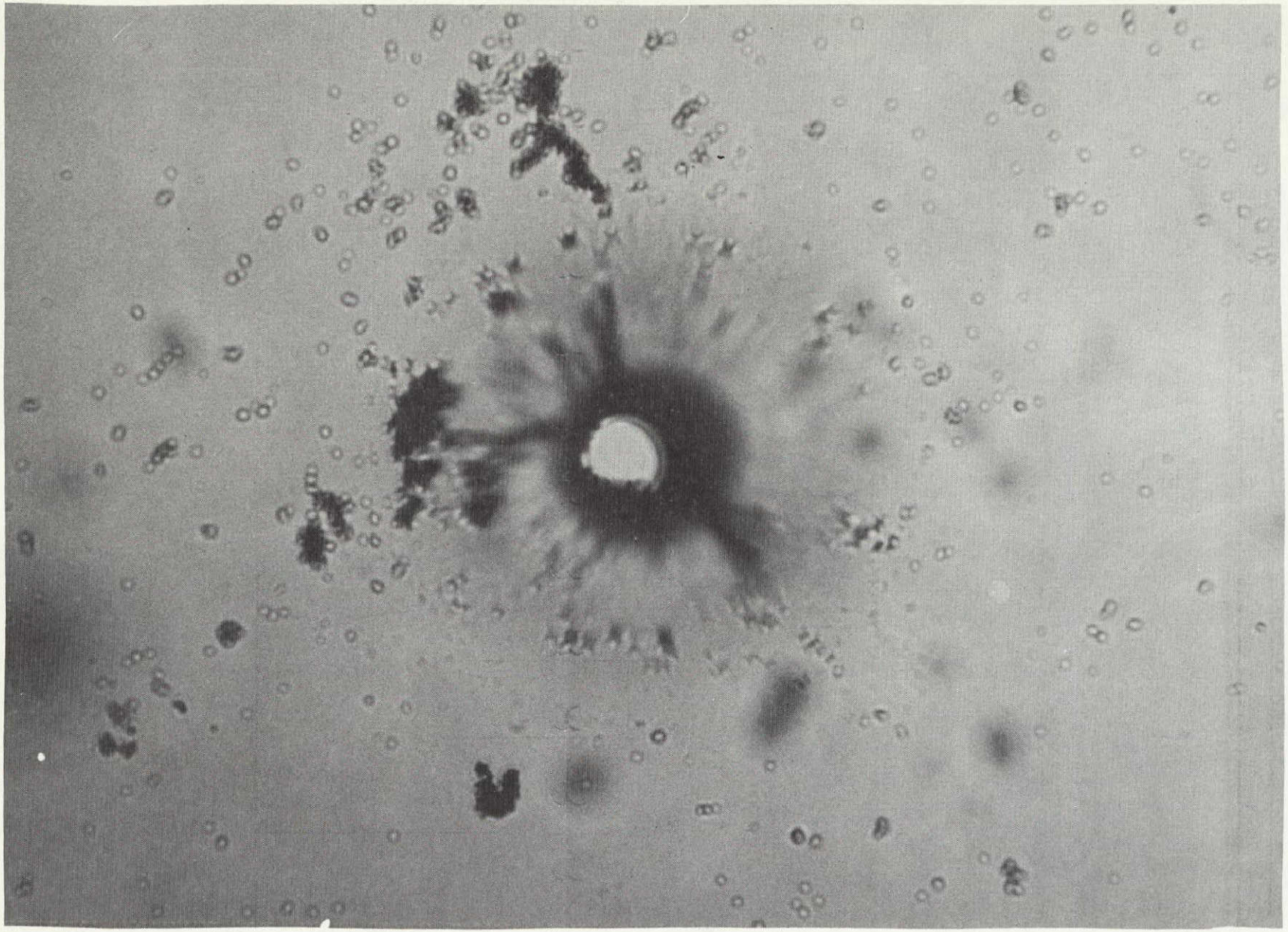


FIGURE 21. - ONE MIL ORIFICE - NO VIOLATION -

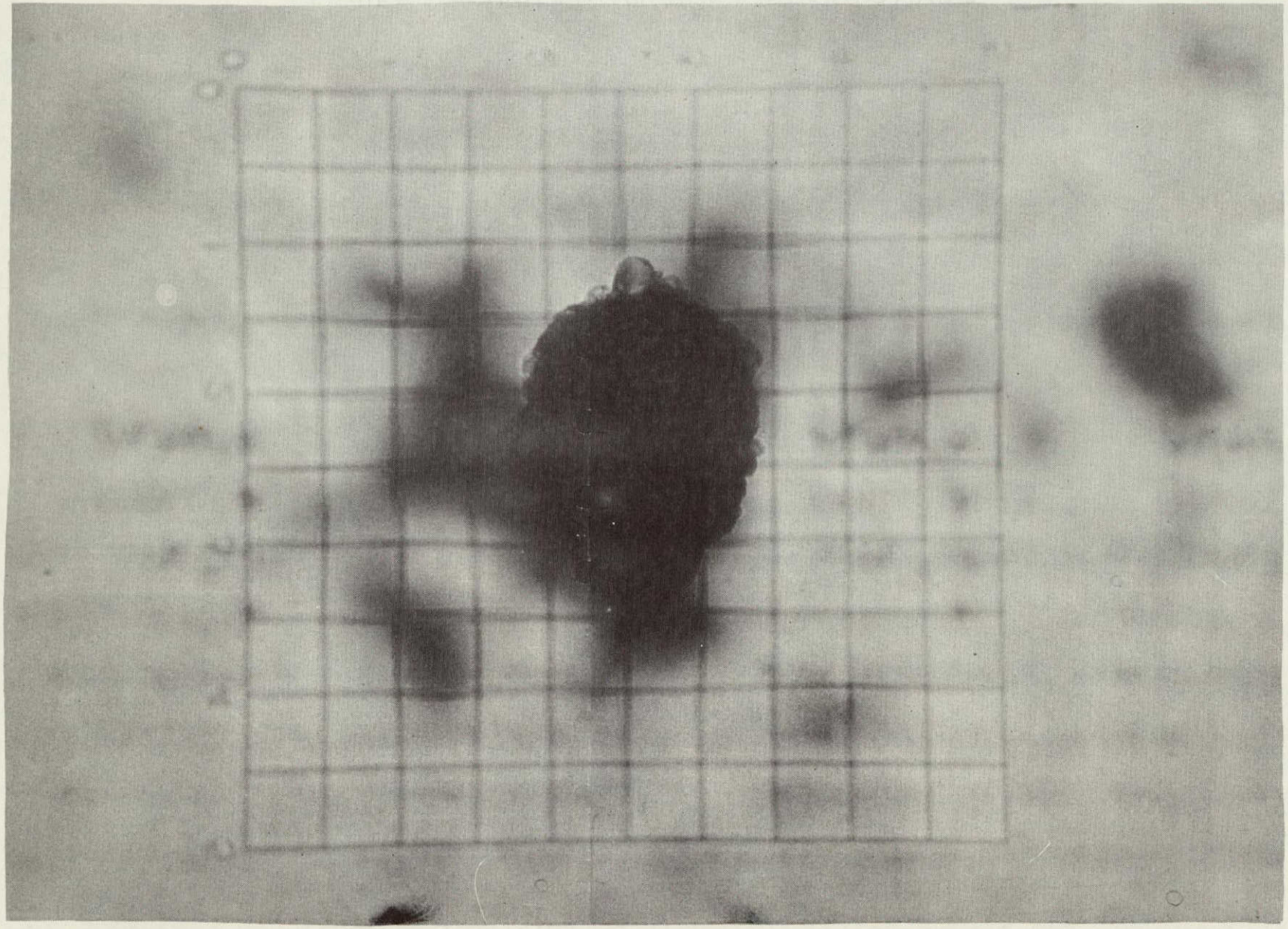


FIGURE 22. - PLUGGED SIX MIL ORIFICE FOLLOWING EIGHT MINUTE REVERSE FLOW TEST-
INSIDE SURFACE OF ORIFICE ΔP = 4 INCHES WATER, MAG. = 80X

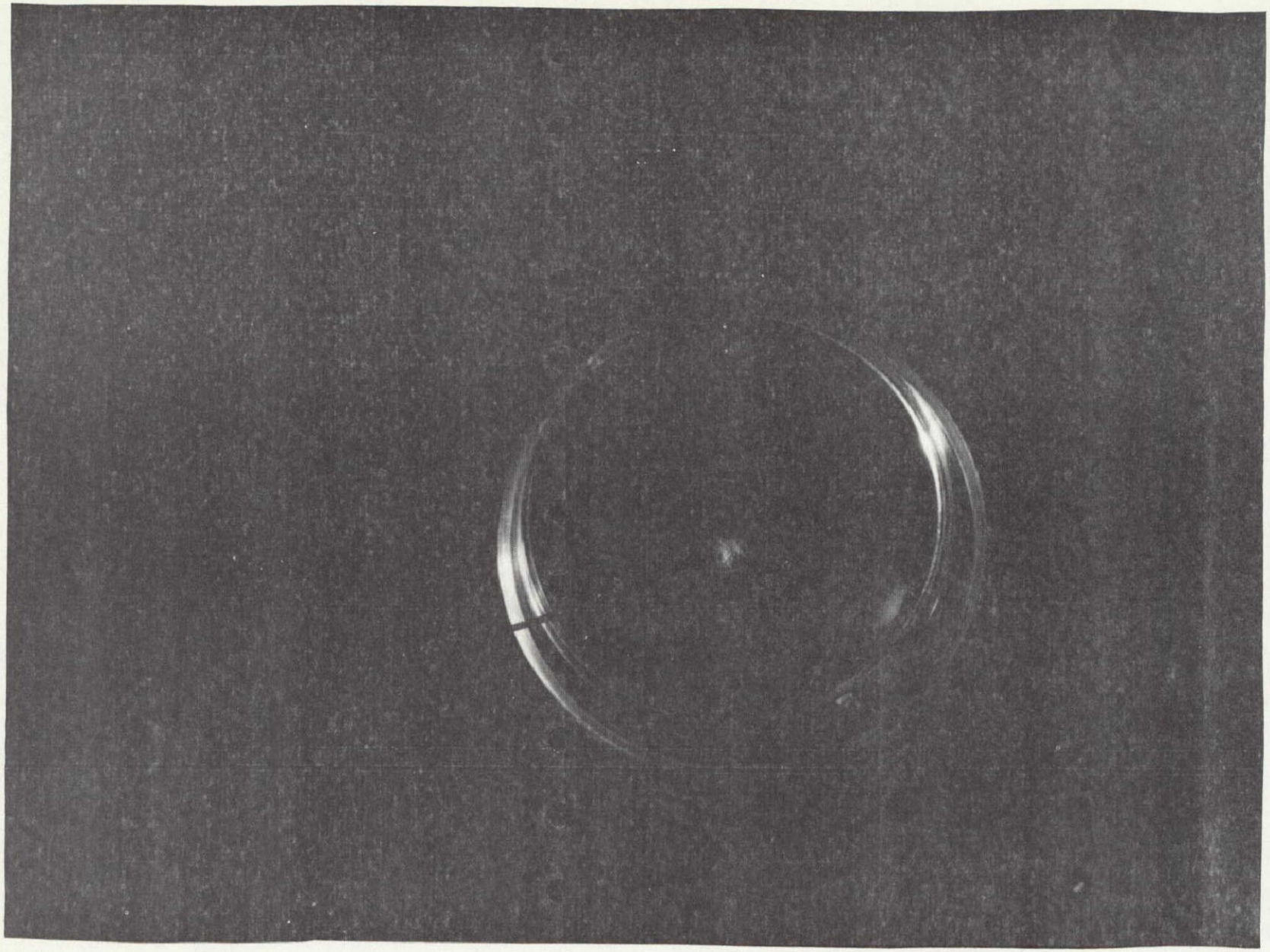


FIGURE 23. - PETRI DISH SHOWING VIOLATION FOLLOWING EIGHT MINUTE REVERSE FLOW TEST - SIX MIL ORIFICE ΔP = 4 INCHES WATER

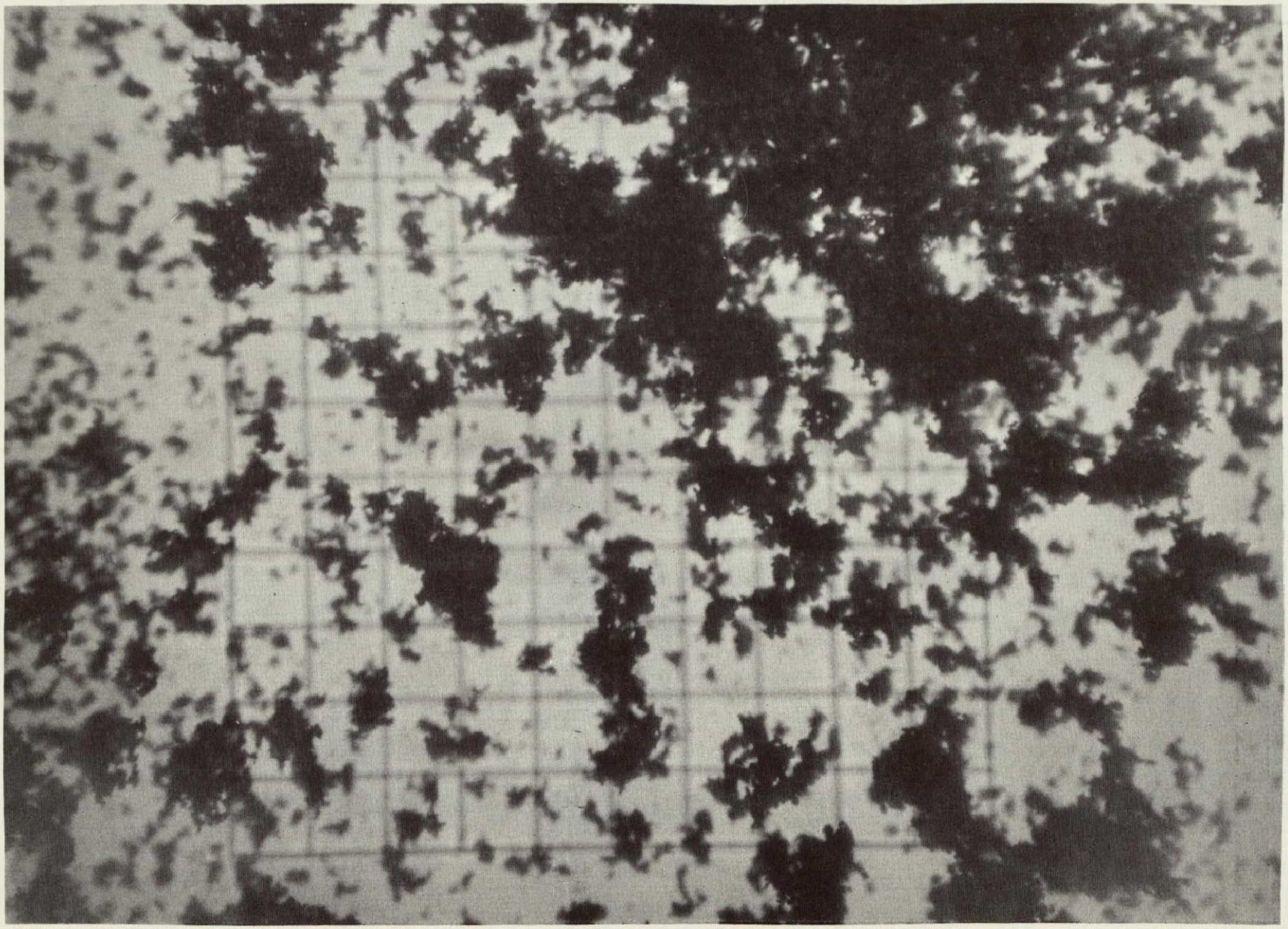


FIGURE 24. - VIOLATION OF SIX MIL ORIFICE FOLLOWING EIGHT MINUTE REVERSE FLOW TEST ΔP = 4 INCHES WATER, MAG. = 100X

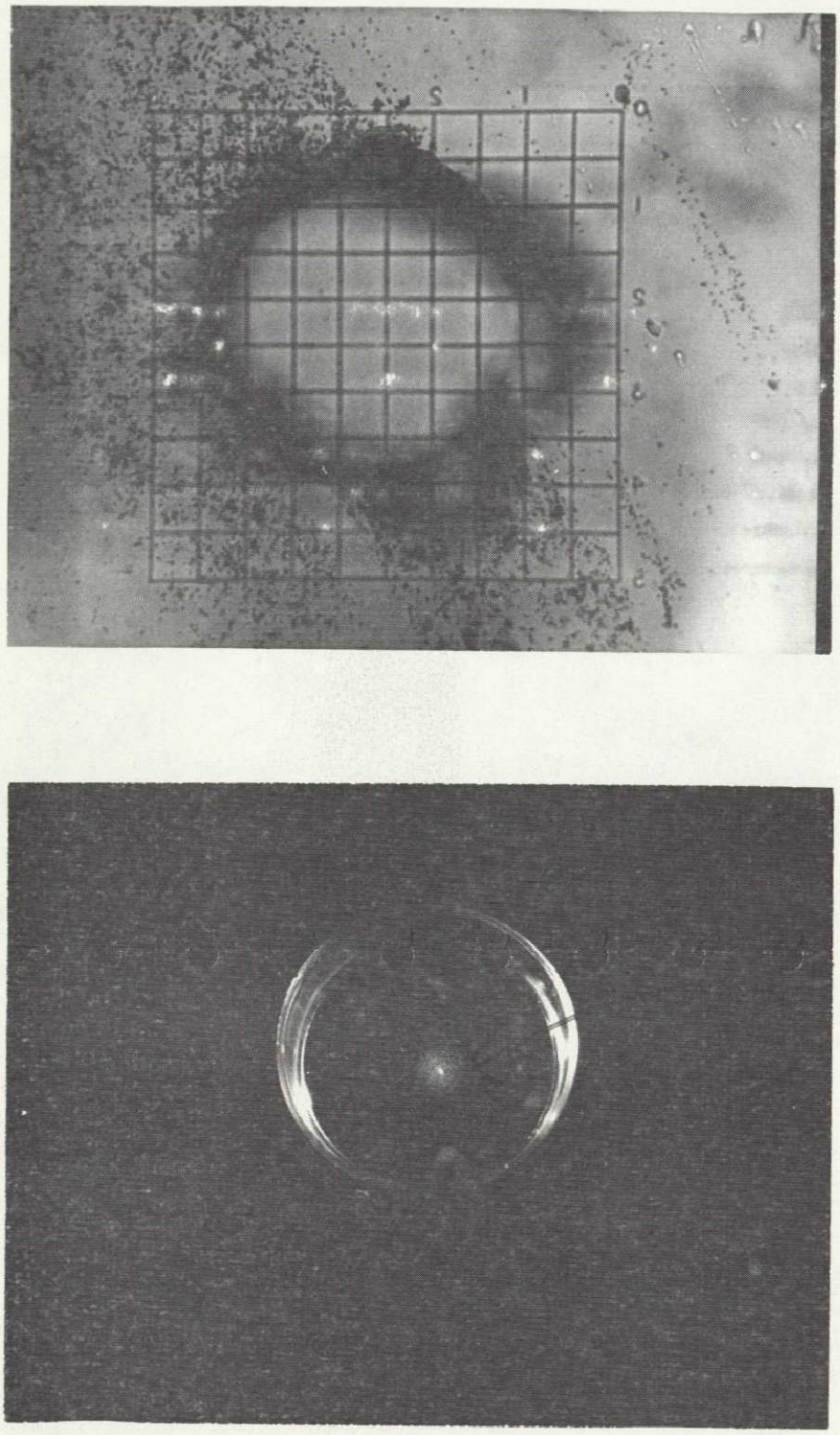


FIGURE 25. - VIOLATION OF TEN MIL ORIFICE
TOP: 100X MAGNIFICATION OF ORIFICE
BOTTOM: PETRI DISH FOLLOWING TEST OF TEN MIL ORIFICE

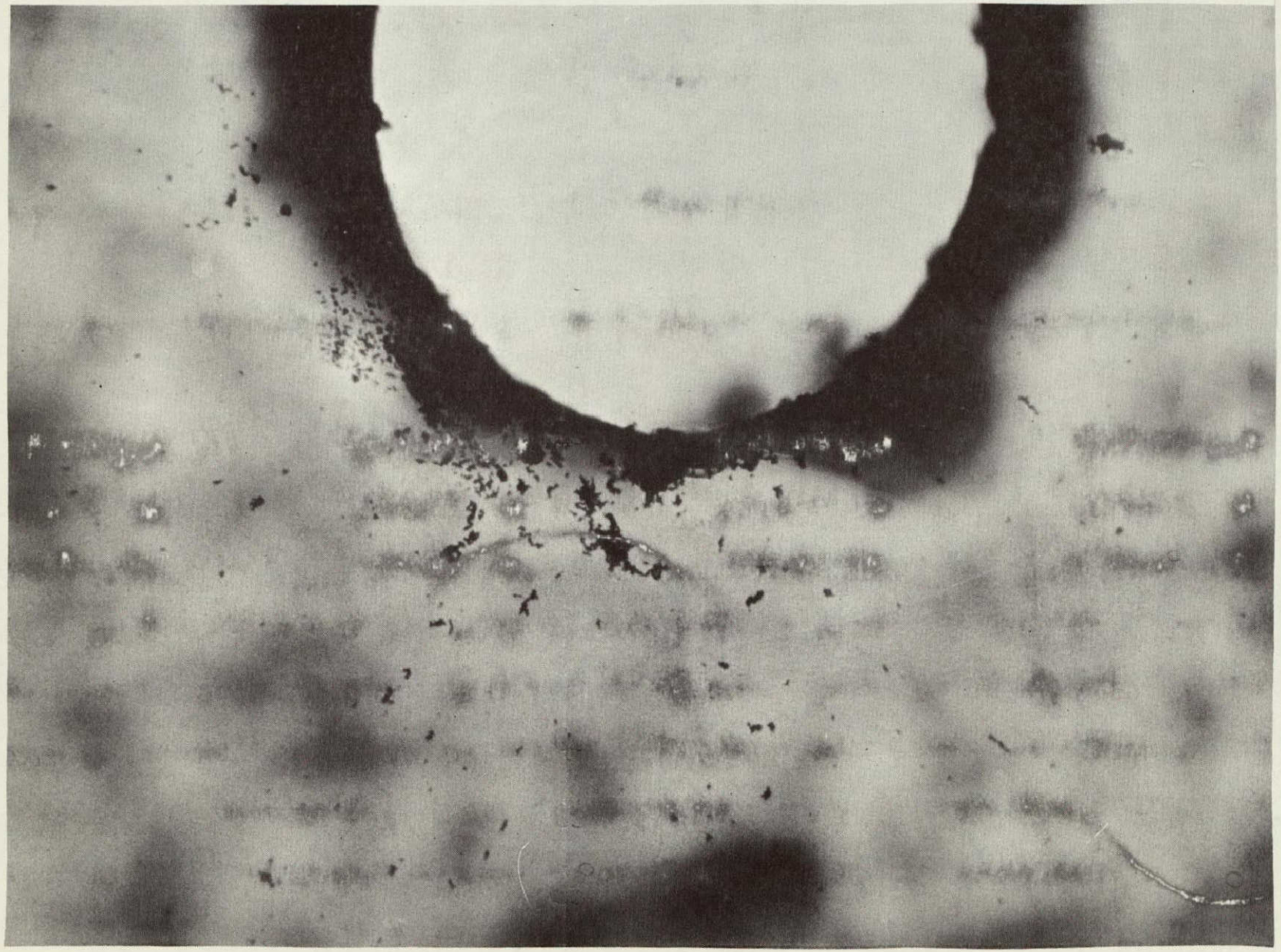


FIGURE 26. - TWO MIL ORIFICE FOLLOWING CONTROL EIGHT MINUTE REVERSE FLOW TEST - NOTE VIOLATION ΔP = 4 INCHES WATER, MAG. = 80X

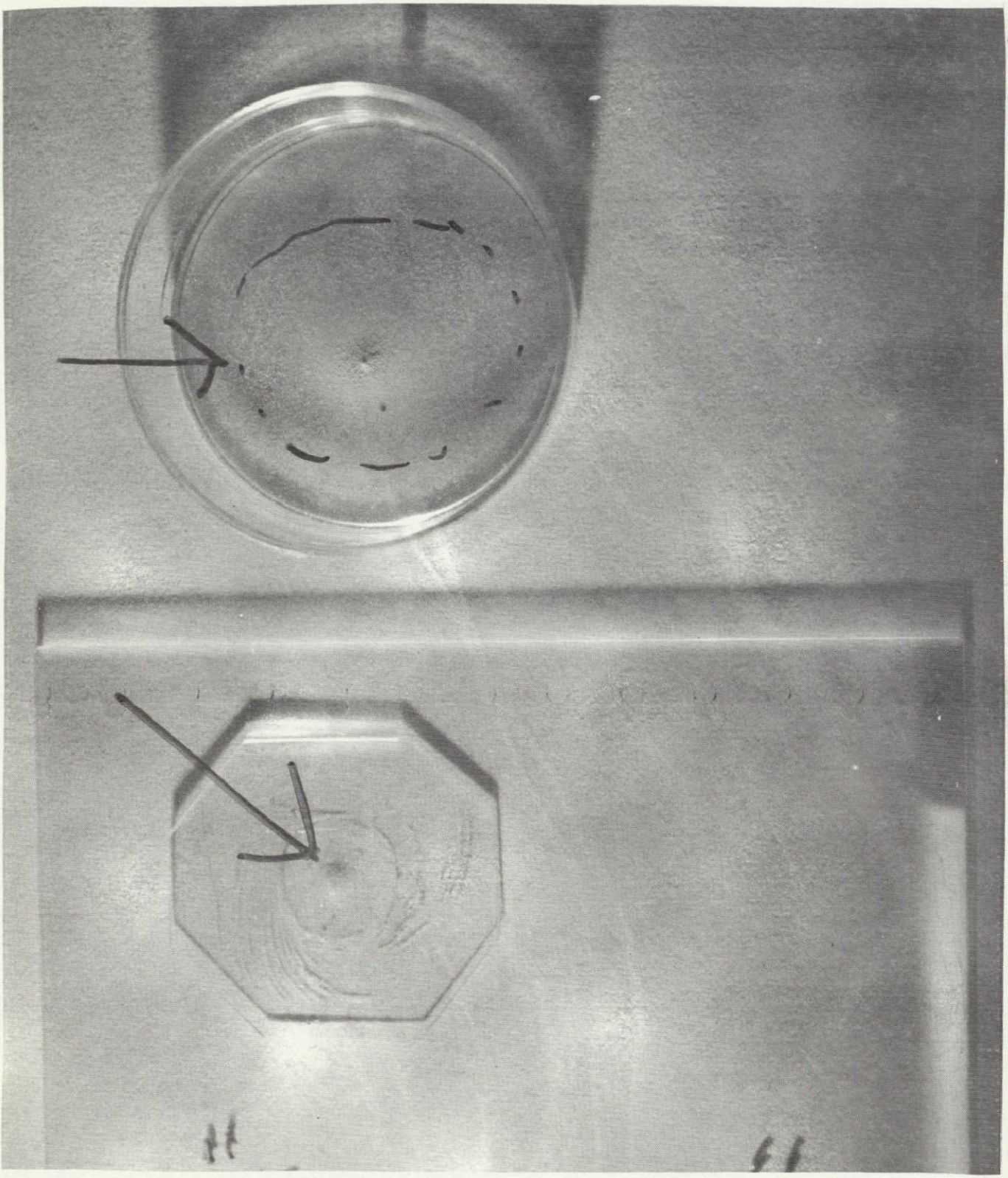


FIGURE 27. - REVERSE FLOW TEST FOR EIGHT MINUTES
WITH 20 MIL ORIFICE ΔP = 4 INCHES WATER
TOP: 35 x 10MM PETRI DISH SHOWING SPORE PILE-UP
BOTTOM: COLLECTION OF SPORES ON ORIFICE COVER SLIP